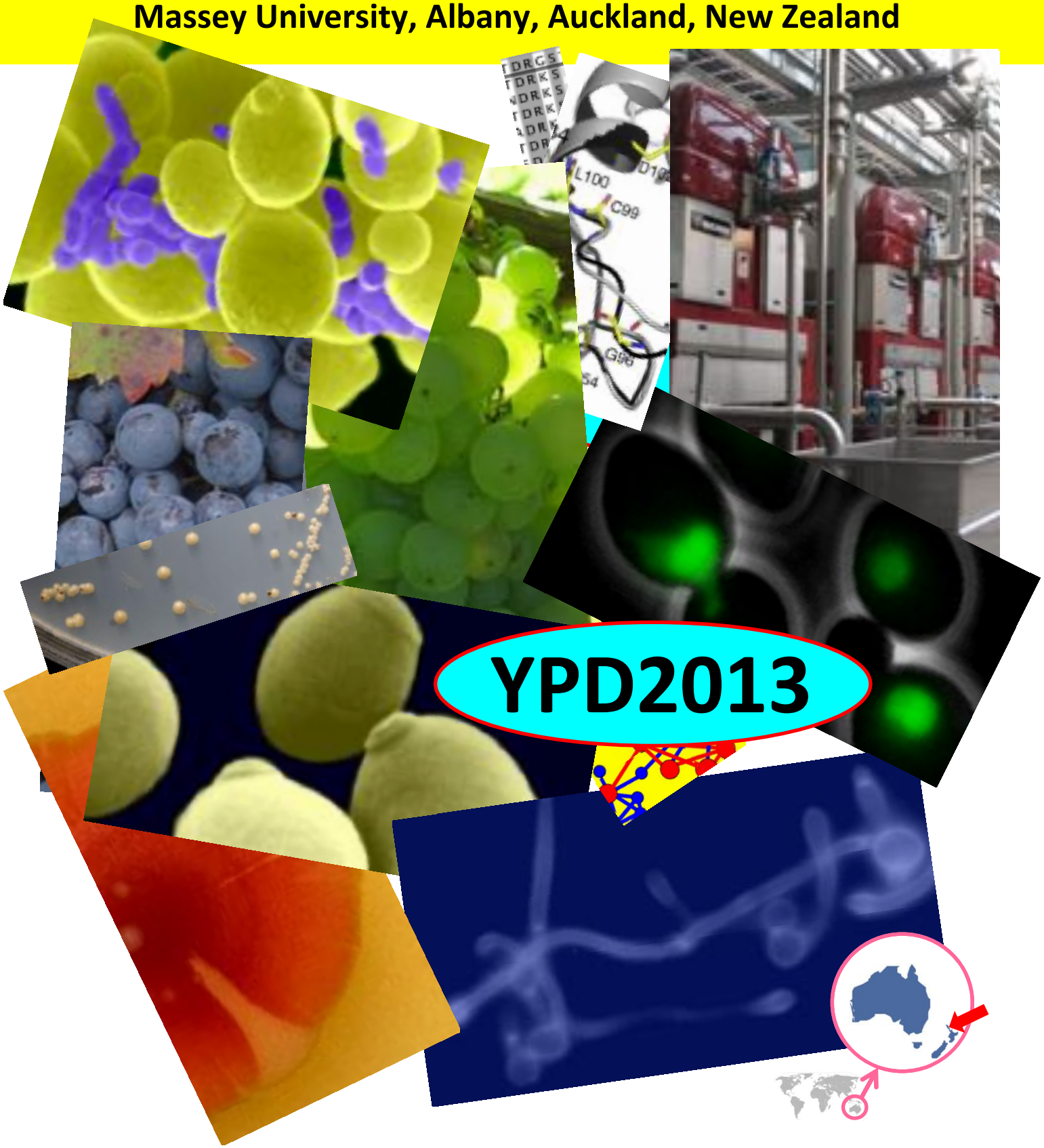
Yeasts: Products, Discovery, and more

23 – 25 November, 2013

Massey University, Albany, Auckland, New Zealand



YPD2013

Yeasts: Products, Discovery, and more

23 – 25 November, 2013, Massey University, Sir Neil Waters Lecture Theatres
State Highway, Gate 1, Albany, Auckland, New Zealand

A meeting of the Australasian Yeast Group, including the Yeast Special Interest Group of the Australian Society for Biochemistry and Molecular Biology (ASBMB), and the New Zealand Microbiological Society (NZMS) Eukaryotic Special Interest Group held at **Massey University, Auckland, New Zealand**, prior to the NZMS Conference in Hamilton, Nov 26-28

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Welcome to the 2013 Yeast Products, Discovery and More … meeting!!

It is our pleasure to welcome you to the Massey University Albany campus for this meeting of the Australasian Yeast Network and the New Zealand Microbiological Society Eukaryotic Special Interest Group.

We are excited about the program we have put together, with a great mix of Australasian and International speakers. We are particularly appreciative of all the International speakers for sparing their time to travel all the way to New Zealand, and to thank our Australian colleagues for making the trip over the ditch. Welcome also to all the yeast researchers from across New Zealand.

We have mixed the scientific program with an opportunity to experience a small part of northern Auckland, as well as some of the products of our hemiascomycetous friends, and hope you will enjoy all these aspects of the meeting.

A very big thank-you to all our sponsors, without whom this meeting would not be possible. We would like to acknowledge Massey University's Institute of Natural and Mathematical Sciences, the Maurice and Phyllis Paykel Trust, and the New Zealand Microbiological Society for major funding. We also thank Singer Instruments for their generous sponsorship of the Vino-Bocconcino evening, and the Australian Society for Biochemistry and Molecular Biology, Formedium, Scientex, Lab Supply, Raylab, Life Technologies, SciMed, Thermo Fisher, Sunrise Science Products, Millennium Science, and Global Science for their support of the meeting. Cheers also to Colleen van Es for taking care of most of the practical side of the meeting organization.

Finally, it is our aim to make this meeting as informal and collegial as possible. Therefore we hope you will all take the opportunity to interact and engage with each other during this meeting in a relaxed setting, and will help us celebrate yeast as a wonderful organism for research!

The 2013 YPD Organising Committee

YPD2013 gratefully acknowledges the invaluable support of the following companies / organisations:

YPD2013 Programme, quick overview

November 23rd

from 8:15	Registration
8:45 – 9:00	Conference opening
9:00 – 9.45	Kevin Verstrepen
9:45 – 10:15	Session 1
10:15– 10:45	*Morning tea*
10.45 – 12:30	Session 2
12.30– 13.30	*Lunch*
13.30 – 14:15	Ana Traven
14:15 – 15.30	Session 3
15:30– 16:00	*Afternoon tea*
16:00 – 16:45	Ed Louis
16:45 – 18:00	Session 4
18:00 -	Singer's Vino-Bocconcino (wine-nibbles) pre-dinner experience
	Session 5: Poster session
– 21:00	Pizza dinner

November 24th

8.30 – 9.15	Yury Chernoff
9.15 – 10:00	Session 6 - student best-speaker competition
10.00 – 10.30	*Morning tea*
10.30 – 12.15	Session 7 - student best-speaker competition
12:15– 13.15	*Lunch*
13.15 – 14:00	Sepp Kohlwein (Session 8)
14:00	*assemble for group picture, front entrance of SNW Lecture Building*
14:00– 14:20	*Quick afternoon tea*
14:30 sharp -	Sightseeing tour, including wine tasting
- ~22:00	Conference Dinner

November 25th

9.00 – 9.45	Alistair Brown
9:45 – 10:00	Session 9
10:00 – 10:30	*Morning tea*
10.30 – 11:00	Session 10
11.00 – 11:45	Romain Koszul
11:45 – 12:30	conclusion of the conference

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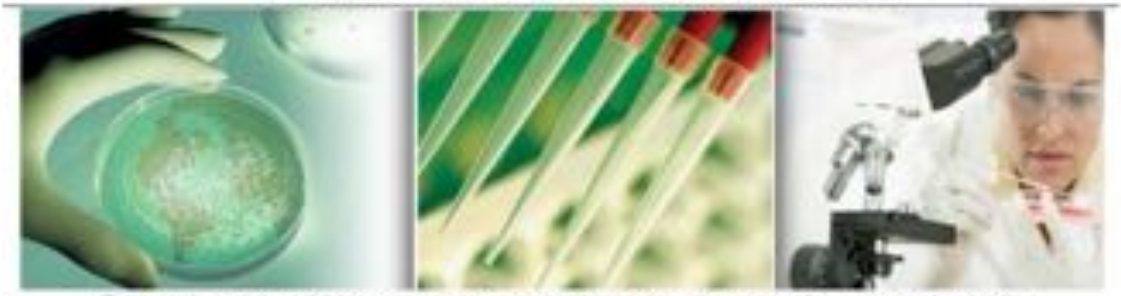

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YPD2013 programme

November 23rd

from 8:15	Registration
8:45 – 9:00	Conference opening

Session 1	
Chair: **Robert Learmonth**, University of Southern Queensland, Australia	
9:00 – 9.45	**Kevin Verstrepen,** Katholieke Universiteit Leuven, Belgium
9:45 – 10:15	**Richard Gardner**, The University of Auckland, New Zealand
	Chien-Wei (Max) Huang, The University of Auckland, New Zealand

10:15 – 10:45	*Morning tea*

Session 2	
Chair: **Jörg Heierhorst**, St Vincent's Institute, Australia	
10.45 – 12:30	**Margarita Santiago**, The University of Auckland, New Zealand
	Tommaso Liccioli, University of Adelaide, Australia
	Thomas Pfeiffer, New Zealand Institute of Advanced Study, NZ
	Jan Schmid, Massey University, New Zealand
	Claudia Vickers, Australian Inst. for Bioengineering & Nanotechnology, Australia
	Elisabeth Walker, University of Adelaide, Australia
	Tatiana Chernova, Emory University, USA

12.30 – 13.30	*Lunch*

Session 3	
Chair: **Andrew Munkacsi**, Victoria University Wellington, New Zealand	
13.30 – 14:15	**Ana Traven**, Monash University, Australia
14:15 – 15.30	**Jörg Heierhorst**, St Vincent's Institute, Australia
	Erwin Lamping, University of Otago, New Zealand
	Andrew Munkacsi, Victoria University Wellington, New Zealand
	Alan Munn, Griffith University, Australia
	Justin M. O'Sullivan, Liggins Institute, New Zealand

15:30– 16:00	*Afternoon tea*

Session 4	
Chair: **Claudia Vickers**, Australian Institute for Bioengineering & Nanotechnology, Australia	
16:00 – 16:45	**Ed Louis**, University of Leicester, UK
16:45 – 18:00	**Rashmi Ramesh**, Massey University, New Zealand
	Robert Learmonth, University of Southern Queensland, Australia
	Richard da Silva, Federal University of Sao Paulo, Brazil
	Joanna Sundstrom, University of Adelaide, Australia
	Matthew Woods, Massey University, New Zealand

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18:00 -	*Singer's Vino-Bocconcino (wine-nibbles) pre-dinner experience*
Session 5	Poster session
	Bede Busby, Victoria University of Wellington, New Zealand
	Rebecca Deed, **Keith Richards**, University of Auckland, New Zealand
	Viviane Jochmann, Massey University, New Zealand
	Samuel Lam, University of Melbourne, Australia
	Su Jung Lee, Massey University, New Zealand
	Julie Nguyen, Monash University, Australia
	Trung Dung Nguyen, University of Adelaide, Australia
	Christina Roberts, Victoria University of Wellington, New Zealand
	Richard da Silva, Federal University of Sao Paulo, Brazil
~19:30 - 21:00	*Pizza dinner*

November 24th

Session 6	
Chair: **Jan Schmid,** Massey University, New Zealand	
8.30 – 9.15	**Yury Chernoff,** Georgia Institute of Technology, USA
9.15 – 10:00	Student speaker competition
	Inswasti Cahyani, Liggins Institute, New Zealand
	Simon Dillon, University of Adelaide, Australia
	Sarah Knight, University of Auckland, New Zealand

10.00 – 10.30	*Morning tea*

Session 7	
Chair: **Ana Traven**, Monash University, Australia	
10.30 – 12.15	Student speaker competition, continued
	Danfeng Long, University of Adelaide, Australia
	Daniella Quintana, Massey University, New Zealand
	Miguel Roncoroni, University of Auckland, New Zealand
	Renuka Shanmugam, Massey University, New Zealand
	Angavai Swaminathan, Monash University, Australia
	Ee Lin Tek, University of Adelaide, Australia
	Jin Zhang, University of Adelaide, Australia

12:15– 13.15	*Lunch*

Session 8:	
Chair: **Ana Traven**, Monash University, Australia	
13.15 – 14:00	**Sepp Kohlwein**, University of Graz, Austria

14:00	Assemble for group picture, front entrance of SNW Lecture Building
14:00– 14:20	*Quick afternoon tea*

14:30 sharp -	Bus tour, to visit:
	Winery for wine tasting
	Muriway beach (Black sand beach, Gannet colony)
	Drop off at the Conference Dinner venue
	Prize giving for the Best Student Speaker
- 21:30	Bus will depart from the dinner venue at 21:30 pm

YPD2013

-Yeasts, Products, Discovery and more

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November 25th

Session 9	
Chair: **Justin M. O'Sullivan**, Liggins Institute, New Zealand	
9.00 – 9.45	**Alistair Brown**, University of Aberdeen, UK
9:45 – 10:00	**Richard Cannon**, Otago University, New Zealand

10:00 – 10:30	*Morning tea*

Session 10	
Chair: **Alan Munn,** Griffith University, Australia	
10.30 – 11:00	**Michael Bolech**, Massey University, New Zealand **Catrin Guenther**, The University of Auckland, New Zealand
11.00 – 11.45	**Romain Koszul**, Institut Pasteur, France
11.45 – 12.30	Conclusion of the conference

Invited Speakers

Invited Speaker

Evolutionary rewiring in a fungal pathogen of humans – tuning adaptive responses to host niches

Alistair J.P. Brown[1]

[1]Aberdeen Fungal Group, School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK.

Candida albicans is a major fungal pathogen of humans. This yeast is a commensal of the skin, gastrointestinal and urogenital tracts, it causes mucosal infections (thrush), and it causes life-threatening systemic infections in immunocompromised patients. Many molecular medical mycologists have been dissecting *C. albicans* virulence with a view to understanding the mechanistic basis for the pathogenicity of this yeast. The community has regularly looked to the *Saccharomyces cerevisiae* paradigm when developing working hypotheses about the regulatory networks that control pathogenicity. However, as the *C. albicans* research field has matured in the post-genomics era, it has become abundantly clear that while the functions of many regulatory modules have been evolutionarily conserved in these yeasts, others have been rewired such that they now drive different cellular processes. Classic examples of transcriptional rewiring include the functional reassignment of Gal4 in carbohydrate assimilation, Rap1 in ribosomal gene regulation, and α2 in the mating circuitry. More recently we have identified the evolutionary rewiring of some post-transcriptional circuitry involved in the control of carbon assimilation in *C. albicans*. This evolutionary rewiring is thought to promote the survival of *C. albicans* in host niches by tuning the expression of the fitness attributes and virulence factors during disease progression.

Thanks to the NZMS for funding the speaker

Invited Speaker

Chaperone machinery of protein-based inheritance

Yury O. Chernoff[1]

[1]School of Biology, Georgia Institute of Technology, Atlanta, GA, USA

Yeast self-perpetuating amyloids (prions) are protein-based heritable elements. Yeast prions provide a valuable model for understanding human amyloid diseases. Prions also control phenotypically detectable traits and influence various aspects of yeast biology. The Hsp104/70/40 chaperone network controls fragmentation, propagation and segregation of yeast prions. Hsp70 chaperones and their Hsp40 partners have also been implicated in a variety of cellular processes, including nascent polypeptide folding and protein trafficking. Together with the disaggregating chaperone Hsp104, Hsp70/40 proteins play a crucial role in stress response. Cochaperones modulate interactions of Hsps with different types of aggregation-prone proteins, thus providing a link between the cellular and prion-related functions of chaperones. Alterations of the ribosome-associated chaperone complex promote de novo prion formation and influence effects of cytosolic Hsps on prion propagation. These results establish a connection between the chaperone roles in nascent polypeptide folding and in formation/propagation of self-perpetuating amyloid aggregates.

Invited Speaker

Keeping membrane lipids in balance: deciphering the metabolic flux of fatty acids

Sepp D. Kohlwein[1]

[1]Institute of Molecular Biosciences, University of Graz, Austria

The mechanisms by which cells establish and regulate the amount and composition of their membranes are poorly understood. A major determinant of membrane lipid synthesis and composition is the availability of fatty acids that are either derived from endogenous synthesis, lipid turnover or from external supply. Excess fatty acids are typically channelled into triacylglycerol (TAG) synthesis, and yeast and mammalian cells lacking the capacity to synthesize TAG are highly sensitive to (unsaturated) fatty acid treatment. Fatty acid-induced lipotoxicity may play a key role in the development of lipid-associated disorders in humans.

We present evidence that shows that the acyl-chain composition of glycerolipids in yeast is to a large extent determined by the activity of the key enzyme of fatty acid synthesis, acetyl-CoA carboxylase (Acc1). Acc1 activity also has profound effects on TAG synthesis, fatty acid sensitivity and gene expression. Acc1 is metabolically highly regulated, which may explain some of the flexibility of the yeast lipidome in growing cells.

Supported by the Austrian Science Funds, FWF, project F3005 SFB LIPOTOX.

Thanks to the Maurice and Phyllis Paykel Trust, and to the Institute of Natural and Mathematical Sciences, Massey University, for funding the speaker

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Invited Speaker

Exploring yeasts genome organizations.

Martial Marbouty, Axel Cournac, Julien Mozziconacci, Romain Koszul[1]

[1]Institut Pasteur, France

Most of our knowledge regarding chromosome organization in yeast comes from the well-studied hemiascomycetes yeast *Saccharomyces cerevisiae*. Several studies have unveiled or confirmed the influence of chromosome organization and dynamics on the regulation of a number of DNA-related processes such as gene expression and DNA repair. In addition, comparative genomics approaches within this phylum explored both adaptive rules and evolutionary pathways leading, for instance, to the diversification of regulatory networks. However, no comparative analysis has been performed so far regarding conservation and differences in genome organization of yeast species. We decided to explore genome organization of five species from the Ascomycota phylum, selected to represent various branches of the lineage or for their interest for humans. Using a combination of biochemical, genomic and modeling approaches, the chromosome organization in various yeast species will be presented. In addition, a new approach for genome assembly and strain resequencing will also be described.

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Invited Speaker

Exploring genetic and phenotypic variation in *Saccharomyces* yeasts

Ed Louis[1]

[1]Centre for Genetic Architecture of Complex Traits, Department of Genetics, University of Leicester, Leicester LE1 7RH

Recent surveys of whole genomes of yeast isolates have revealed a great deal of genetic variation including new gene families that don't exist in the lab strains. In addition, phenotypic variation broadly correlates with the global genetic variation. Specific combinations of genetic variants are limited to distinct populations, which may be adapted to their respective niches. Breeding between these isolates generates genetic combinations never before seen in nature and these result in phenotypic diversity beyond what is seen in extant populations. This may explain the paradox of relatively low genetic diversity but high phenotypic diversity in *S. cerevisiae* compared to *S. paradoxus*. Some observations arising from breeding include: transgressive variation beyond that of the parental population, antagonistic QTLs whose effect is opposite to that expected from the parental origin, and linked QTLs of mixed effects. Using classical breeding and modern genomics we can determine the underlying genetics of many quantitative traits at high resolution and sensitivity, as well as create strains with desired and possibly novel phenotypes. This approach can be combined with genetic modification to engineer novel pathways as well as with experimental evolution to improve traits.

Thanks to the Institute of Natural and Mathematical Sciences, Massey University, for funding the speaker

Invited Speaker

Mitochondria, cell walls and biofilms: is there a link in *Candida*?

Yue Qu[1,2], Miguel Shingu-Vazquez[1], Julie Nguyen[1], Jiyoti Verma-Gaur[1], Victoria L Hewitt[1,2] and Ana Traven[1]

[1]Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800, VIC, Australia (ana.traven@monash.edu)
[2]Department of Microbiology, Monash University, Clayton 3800, VIC, Australia

Mitochondria are central metabolic organelles in eukaryotes. As such, mitochondria play vital roles in many cellular pathways, and their biogenesis and functions are integrated into the regulatory networks that control cell physiology on a global level. Recent work has shown that changes to mitochondrial function can have a profound impact on pathways important for virulence of human fungal pathogens, for example *Candida albicans* and *Cryptococcus neoformans*. Importantly, there are several mitochondrial factors that lack close homologs in animals, and could provide promising targets for future antifungal drug development. Our work in *C. albicans* has shown that mitochondria impact on cell wall integrity and the susceptibility to the echinocandin class of antifungal drugs. We have identified mitochondrial factors important for fitness and virulence of *Candida* that lack close homologs in humans, such as subunits of the ERMES (ER-Mitochondria Encounter Structure) and SAM (Sorting and Assembly Machinery) complexes. Our current work is aimed at understanding the cellular functions of these complexes using *C. albicans* as a model system. We are also addressing the contribution of metabolic reprogramming and mitochondrial function to the growth, maturation and drug susceptibility of multicellular communities, such as *C. albicans* biofilms.

Thanks to Thermo Fisher Scientific and Sunrise Science Products
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Saccharomyces cerevisiae is a powerful model for understanding genetic regulation in eukaryotic cells. Thermo Scientific yeast resources include multiple collections for interrogating the yeast genome, including Tagged ORFs, Knockout Strains, Protein-Protein Interaction Collections and Mutant Strains and Screening Collections. See which range of yeast collections apply to your research below from Thermo Scientific, to provide you with effective, high quality tools to fast track your results.

Knockout Strains

Yeast Collection

The Saccharomyces Genome Deletion Project[1] created over 6,000 gene-disruption mutants* that cover 96% of the yeast genome providing a unique tool for functional analysis. Molecular barcodes that identify each strain allows phenotypic analysis to be performed on a single gene basis or on a genome-wide scale.[2]

Availability Table

Available Yeast Knockout Strain Collections	Catalogue N°
Yeast Knock Out MATa Collection	YSC1053
Yeast Knock Out MAT alpha Collection	YSC1054
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Yeast Knock Out Homozygous Diploid Collection	YSC1056
Yeast Knock Out Essential Genes Collection	YSC1057
Yeast Knock Out Strain	YSC1021

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	Yeast TAP-Fusion Membrane Protein Subset	YSC1247
	50 µg Rabbit Anti-TAP Antibody	OAB1051
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	Yeast Tet promoters Hughes (yTHC) Strain	YSC1180
	Yeast Tet promoters Hughes Parental Strain R1158	YSC1210
Tagged ORF Clones and Strains	Yeast ORF Collections	YSC3867, YSC3868, YSC3869, YSC3870, YSC3880
	HA-tagged Collections	YSC1068, YSC1070, YSC1764
	GST-tagged Collections	YSC4575, YSC4423
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Yeast Decreased Abundance (DAmP)	Haploid DAmP Strains & Collections	YSC5090, YSC5094
	Diploid DAmP Strains & Collections	YSC5050, YSC5083
Genomic DNA	Genome Tiling Strains & Collections	YSC4413, YSC5103, YSC5034
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Invited Speaker

How do new genes evolve?

Kevin J. Verstrepen[1]

[1]KU Leuven Laboratory for Genetics and Genomics and VIB Laboratory of Systems Biology, Gaston Geenslaan 1, B3001 Leuven, Belgium.
(Kevin.Verstrepen@biw.vib-kuleuven.be)

Gene duplications are an important source of evolutionary innovation because the new gene copy can evolve novel functions. However, the molecular mechanisms that underlie the neofunctionalization of such newly duplicated paralogs remain poorly defined. We have previously reported the reconstruction of ancient maltase enzymes to unravel how, after duplication of the ancestral gene, a few simple point mutations in one paralog shifted the function towards a new set of substrates (Voordeckers et al, 2012). A next question that we are currently investigating is how duplication of a gene encoding a transcriptional regulator aided in the development of a new regulatory cascade that ensures activation of the proper maltase paralog in response to the two different substrate categories, and how the regulation of these metabolism genes is tuned to different environmentsn.

Similarly, duplication of parts of coding regions lead to the formation of tandem repeats within genes. In contrast to duplications of entire genes, such internal duplication events do not generate novel functions, but rather aid to tune the existing function to changing environments (Verstrepen 2005, Vinces 2009, Gemayel 2011). Here, we specifically focus on CAG repeats that are often found within regulatory genes. We show that variation in these repeats result in changes in the activity of the transcription factor, which in turn yields different levels of transcription of the target genes of the regulator.

Oral and Poster Presentations

Presenting Authors in Alphabetical Order

Cell density impacts *Candida glabrata* survival in hypo osmotic stress

Hala Almshawit[1], Dodie Pouniotis[1] and Ian Macreadie[1]

[1]School of Applied Sciences, RMIT University, Bundoora, VIC, Australia.

Candida glabrata cells suspended in water are under hypo osmotic stress and undergo cell death in 1-2 days, unless they are at a density of more than 10^5 CFU/ml. The dying cells exhibit Annexin V staining, a primary indicative of programmed cell death (apoptosis). In a higher cell density cells are protected and survive at least four days. Filtrates from cells at high density can protect those at lower density, indicating that cells release substances, amounting to ~5 mg/litre of cell suspension, that protect each other against hypo osmotic stress. In a concentrated form the released materials can support growth, indicating that the protective material includes carbon and nitrogen sources, as well as vitamins that required by *C. glabrata* for growth. We conclude that cell death from hypo osmotic stress can be alleviated by osmotic protection or by small amounts of nutrients.

Other

Identifying potential Yih1-binding proteins required for stimulating protein kinase Gcn2.

Michael Bolech[1], Mathias Joachim[1,2], and Evelyn Sattlegger[1]

[1] Institute of Natural and Mathematical Sciences, Massey University, Auckland, NZ.
[2] Hamburg University of Applied Sciences, Hamburg, Germany

One of the important control points in intracellular signalling pathways is the protein **Gcn2**, found in all eukaryotes. Under amino starvation conditions Gcn2 is activated and phosphorylates the translation initiation factor eIF2α. This leads to a downregulation of the general proteins synthesis and an upregulation of the synthesis of proteins that are involved in helping the cell overcome the starvation. Gcn2 plays important roles in, for example, memory formation or feeding behaviour, and aberrant Gcn2 function has been implied in many diseases such as cancer or obesity. For this reason it is important to understand how Gcn2 is regulated. However, only few Gcn2 regulators have been identified so far. One positive regulator is Gcn1 to which Gcn2 must bind to become activated. One known negative regulator is IMPACT in mammals, or its orthologue **Yih1** in yeast. IMPACT/Yih1 inhibits Gcn2 activity by competing with Gcn2 for Gcn1 binding. IMPACT/Yih1 itself must be regulated to ensure that it only inhibits Gcn2 when required; however, so far this is not well understood. In yeast, actin has already been identified as a Yih1-binding and regulating protein and modelling exercises strongly suggest that additional proteins bind and regulate Yih1.

The aim of this project is to identify new proteins which bind Yih1 and to first screen for those that are required for Gcn2 activation. To identify Yih1-binding proteins *in vivo*, we overexpressed in yeast cells GST-tagged Yih1 in order to drive weak or transient interactions (characteristic for regulatory protein-protein interactions) by mass action. Yih1 was precipitated via the GST tag and proteins associated with Yih1 identified via mass spectrometry. In addition to known Yih1-binding proteins, we found several additional proteins that were associated with Yih1. Rather than conducting a large range of protein-protein interaction assays to verify the mass spectrometry data, we set out to first screen the Yih1-binding proteins for those that promote Gcn2 function. If a protein is involved in Gcn2 activation, then deleting the respective ORF should result in a growth defect under amino acid starvation conditions and reduced eIF2α phosphorylation levels (eIF2α-P).

The respective strains were retrieved from the yeast deletion collection, and our screen uncovered twenty-one deletion strains that were unable to grow on starvation medium. For four of those, this was associated with impaired increase in eIF2α-P levels. Of those four, so far the growth defect of the *spc72Δ* strain was reversed by a plasmid from the Yeast Genomic Tiling Collection that harbours the *SPC72* gene, suggesting that **Spc72** may be required for promoting Gcn2 function.

Genetic network variation between individuals of statin drugs

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Network biology and pharmacology are rapidly emerging fields and have been developing in a number of eukaryotic model organisms. *Saccharomyces cerevisiae* is one such model, whose genes are highly conserved to humans and given that it was the first eukaryote to be sequenced there are wide range of functional genomic tools available. Network analysis can explain why the majority of single gene knockouts exhibit little or no effect on phenotype by suggesting that naturally occurring biological networks are rich in redundancy and are therefore able to buffer/compensate for the effect of genetic or small molecule perturbation at the single gene level. Our previous work utilised synthetic genetic array analysis and chemical genetic profiling to investigate the pleiotropic effects of the statin family of drugs and their target genes. Although statins are among the most widely prescribed of all drugs, they are not without unwanted side effects that differ in individuals (e.g. muscular myopathy) whose mechanisms remain unknown. We plan to measure and compare genome-wide genetic and chemical genetic interaction in a panel of natural yeast strains that have been fully sequenced and serving as a model for “individuals”. We have screened the panel of strains for variation in drug hypersensitivity and resistance. To create genetic interaction networks the natural strains will be placed on the yeast deletion mutant array genetic background. Genes showing network changes relative to statin sensitivity will then be mapped based on chromosome location and linkage analysis and the changes compared across strains sowing different statin sensitivities.

Do chromosomal interactions with the nucleolus form part of a feedback regulatory loop in *Saccharomyces cerevisiae*?

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Eukaryotic chromosomes are organized within the nucleus such that functional elements (e.g. telomeres, centromeres, and the ribosomal DNA) cluster within a complex three-dimensional network. These network connections are non-random and are hypothesized to be functional. We previously identified one interaction linking the *Saccharomyces cerevisiae RPA135* gene locus[1], encoding the second largest sub-unit of RNA Polymerase I (Pol I), with nucleolar intergenic spacer regions (IGS1). This interaction was intriguing because Pol I is responsible for the production of the 35S transcript ribosomal RNAs, positioned adjacent to the IGS1 locus. We hypothesized that this interaction is regulatory, linking nucleolar transcription with nucleolar structure.

Quantitative Chromosome Conformation Capture (q3C) was used to determine the level of interactions in strains containing incremental replacements (~75bp) of the *RPA135*-tK(CUU)P intergenic region. Replacements were achieved by homologous recombination with a λ-DNA sequence fused to a Kanamycin marker. Seven viable strains were produced; the largest replacement still maintained 260bp of the *RPA135* promoter directly upstream of the ORF. We also assayed interactions in strains having different numbers of rDNA repeats[2], an E-pro mutant and null mutants of Polymerase II transcription factors.

The *RPA135*-IGS1 interaction was dependent on the number of rDNA repeats. In strain with 20-copy of rDNA, interaction frequency was almost abolished (~18%) while 80-copy strain did not differ significantly to wild-type. Interaction was also sequence-driven as replacing the *RPA135*-tK(CUU)P intergenic region reduced the interaction frequencies to ~50% of the wild type level in the replacement mutant strains. Moreover, deleting known transcription factors known to bind to the driving sequence also altered the interaction levels. Interestingly, in some of the replacement and transcription factor mutants, the changes in interaction frequencies were also mirrored by changes in rDNA repeat number. However, *RPA135* transcript levels were maintained at wild-type level in all viable replacement and rDNA copy-number mutants. These results suggest that the *RPA135*-IGS1 interaction may be involved in the regulation and/or stabilization of rDNA copy number.

Heterologous expression of human ABCB5 in *Saccharomyces cerevisiae*

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ATP-binding cassette (ABC) transporters play important roles in many human diseases. ABCB5, which has high (73%) homology to ABCB1 (P-glycoprotein), is highly expressed in melanoma cells, and may contribute to the extreme resistance of melanomas to chemotherapy by efflux of anti-cancer drugs. ABCB5 is unusual in that the major transcripts expressed in melanomas and other cancers (designated ABCB5-α and ABCB5-β isoforms) do not contain the complete domains required for either a full ABC transporter or a typical half-transporter. Thus it is difficult to assign transporter function to the unconventional structures of the ABCB5-α and ABCB5-β isoforms. Our goal was to determine whether we could functionally express various forms of human ABCB5 in *Saccharomyces cerevisiae*, in order to confirm an efflux function for ABCB5 in the absence of background pump activity from other ABC transporters. DNAs encoding ABCB5 sequences were cloned into the chromosomal *PDR5* locus of *S. cerevisiae* ADΔ, a strain in which seven endogenous ABC transporters have been deleted. Protein expression in the yeast cells was monitored by immunodetection using both a specific anti-ABCB5 antibody and a cross-reactive anti-ABCB1 antibody. ABCB5 function in recombinant yeast cells was measured by determining whether the cells possessed increased resistance to known pump substrates, compared to the host yeast strain, in assays of yeast growth. Three ABCB5 constructs were made in yeast. One was derived from the ABCB5-β mRNA, which is highly expressed in human tissues but is a truncation of a canonical full-size ABC transporter. Two constructs contained full-length ABCB5 sequences: either a native sequence from cDNA or a synthetic sequence codon-harmonised for *S. cerevisiae*. Expression of all three constructs in yeast was confirmed by immunodetection. Expression of the codon-harmonised full-length ABCB5 DNA, but not the truncated ABCB5-β, conferred increased resistance, relative to *S. cerevisiae* ADΔ, to the putative substrates rhodamine 123 (R123), daunorubicin, tetramethylrhodamine (TMR), FK506 or clorgyline. Thus full-length ABCB5 can be functionally expressed in *S. cerevisiae*, and it may be possible to use *S. cerevisiae* strains expressing ABCB5 to screen for ABCB5 inhibitors.

Proteolytic processing of the actin assembly protein Lsb1 is stress dependent and modulates aggregation of other proteins

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Yeast prions are self-perpetuating QN-rich amyloids that control heritable traits and serve as a model for mammalian amyloidosis. Propagation of the yeast prion *PSI*$^{+}$ in cell division under normal conditions and during the recovery from damaging environmental stress, depends on cellular chaperones and is influenced by ubiquitin proteolysis and actin cytoskeleton. We demonstrate that destabilization of *PSI*$^{+}$ by short-term heat-shock is increased in the absence of actin assembly protein Lsb1. Heat-shock induces proteasome-dependent processing of Lsb1, which is required for its role in prion maintenance. Processing, ubiquitination and Guided Entry of Tail-anchored proteins (GET) pathway regulate Lsb1 trafficking between the actin patches, endoplasmic reticulum and cytoplasm. Our findings identify actin cytoskeleton protein Lsb1, as a part of a rapid tightly regulated cellular system for control of protein aggregation in changing environments.

Improvement of commercial wine strains for Sauvignon blanc fermentation through backcrossing

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Backcrossing strategies have been successfully utilised to introduce desirable traits in crops for agriculture. At the University of Auckland, we are employing a non-genetically modified backcrossing programme to introgress desirable traits for winemaking in *Saccharomyces cerevisiae*.

Our goal is to produce *S. cerevisiae* offspring with improved Sauvignon blanc fermentation performance and a reduction in undesirable aroma compounds, compared to the parental strains. The first recipient parental strain used for backcrossing, WRS, was derived from a pool of 48 commercial wine yeast strains that had been subjected to repeated rounds of selection at decreasing temperatures (28°C, 18°C, 15°C and 12°C), exposure to sulfite and mating. Two further desirable alleles were then introduced into this parent in a first round of backcrossing: $IRC7^{450}$, encoding a β-lyase for increased production of the volatile thiol 4MMP (boxwood aroma), and *pad1*, a variant of *PAD1* that does not result in the production of phenolic off-flavours (clove aroma). A second round of backcrossing was then used to introduce low H_2S production, as well as a block of novel telomeric genes into the WRS recipient over three rounds of backcrossing.

Three of the resulting introgressed progeny containing the desired markers were fermented at low temperature (15°C) in Chardonnay and Sauvignon blanc juices. All three isolates finished fermenting with fermentation rates similar to, or faster than the parental WRS line. Sensory analysis of the finished wines using a Davis score sheet indicated that the quality of these wines was either no different or higher than the wines produced by the WRS parent.

We have also bred a new homozygous yeast, TB-A-A3, that shows exceptional finishing capability in the cold. We are making further improvements to this second line by backcrossing in a similar set of genes to that described for WRS. We hope to use these superior strains in commercial winemaking trials.

Breeding better wine yeasts

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The improvement of industrial yeast strains has proceeded primarily via selection of natural strains with useful characteristics, in combination with the use of mutagenesis and some small-scale hybridization experiments. Yet *Saccharomyces cerevisiae*, along with a number of other yeast species with industrial applications, possesses biological attributes that make it an excellent prospect for improvement by standard breeding methods. Such breeding approaches have been enormously successful in the improvement of plants and animals, and I believe offer huge potential for the improvement of industrial yeast strains. I will describe results from my lab, which has been applying strategies of selective breeding, combined with the use of molecular markers, to the improvement of white wine yeasts; in particular we are focused on the development of cold-tolerant strains with improved aroma characteristics for Sauvignon blanc.

There are differences in the chemistry and sensory differentiation in red and white wines made by wild yeast fermentation.

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The main characteristics of a 'wild' fermentation are that they are dominated initially by many species of yeast which start the fermentation, but quickly become secondary to *Saccharomyces* species which then dominate (Heard & Fleet 1985). The use of 'wild' yeasts may allow the local nuances of the wine region remain and may give some sensory differentiation (Callejon *et al.* 2010).
'Wild' ferments were conducted alongside *Saccharomyces* inoculated ferments over 3 vintages and under the same conditions using the same starting juice.
The results show differences in wine colour, sensory properties, organic acid profiles and some fermentation volatiles. The differences are statistically significant within years and between inoculated wines and 'wild' wines.
Results from Shiraz wines indicate an increase in anthocyanins, wine colour and non-coloured phenolics for 'wild' wines may be enhanced compared to inoculated wines.
Differences in organic acids are evident over all years of the study. The concentrations of acetic acid in 'wild' wines did not exceed the sensory detection level and were not significantly different from inoculated wines in any year.
Sensory differences in Viognier wines in 2012 and 2013 were significant, with most assessors preferring the wild wines which tended to have a more developed character.

Other

Does chemical attraction drive the coevolution of yeasts and fruit flies?

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Organisms do not just adapt to environments, they may also manipulate them. The yeast *Saccharomyces cerevisiae* engineers its ecosystem by fermenting sugars in fruit to produce a toxic cocktail of alcohol, heat, and carbon dioxide that sabotages microbial competitors. Fermentation by yeast also produces volatile compounds that impart flavour to ferment products such as wine, but the biological function of these compounds is unclear. We are investigating whether ecosystem engineering is a new and previously unstudied mechanism for the evolution of mutually beneficial relationships between species: Yeasts benefit from volatile production because attracting vectors allows them to 'hitch a ride' with flies and escape ephemeral fruits, and flies benefit from following yeast volatiles because they reproduce better in fermenting fruits. In laboratory and field experiments, we found population variance for attractiveness of *Saccharomyces cerevisiae* to female *Drosophila simulans*, and that attractive yeasts were more successfully dispersed than unattractive yeasts. Using Gas Chromatography-Mass Spectrometry we are currently identifying the classes of volatiles produced by yeast during fermentation to see which of those are especially attractive/repulsive to *Drosophila* species. Our results support the hypothesis that ecosystem engineering could drive mutualistic interspecies interactions.

Molecular basis of the essential S phase function of the Rad53 checkpoint kinase

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The essential yeast kinases Mec1 and Rad53, or human ATR and Chk1, are crucial for checkpoint responses to exogenous genotoxic agents, but why they are also required for DNA replication in unperturbed cells remains poorly understood. Here we report that, even in the absence of DNA damaging agents, the *rad53-4AQ* mutant lacking the N-terminal Mec1 phosphorylation site cluster is synthetic lethal with a deletion of the *RAD9* DNA damage checkpoint adaptor. This phenotype is caused by an inability of *rad53-4AQ* to activate the downstream kinase Dun1, which then leads to reduced basal dNTP levels, spontaneous replication fork stalling, and constitutive activation of - and dependence on - S phase DNA damage checkpoints. Surprisingly, the kinase-deficient *rad53-K227A* mutant does not share these phenotypes, but is rendered inviable by additional phospho-site mutations that prevent its binding to Dun1. The results demonstrate that ultra-low Rad53 catalytic activity is sufficient for normal replication of undamaged chromosomes as long as it is targeted towards activation of the effector kinase Dun1. Our findings indicate that the essential S phase function of Rad53 is comprised by the combination of its role in regulating basal dNTP levels and its compensatory kinase function if dNTP levels are perturbed.

Identification of yeast genes responsible for low H_2S production in wine

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The undesirable, rotten-egg odour of hydrogen sulfide (H_2S) produced by yeast is known to reduce sensory quality of the wines and presents an important problem for the global wine industry.

In this project, 192 progeny of two crosses between commercial wine yeast strains, F15xM2 and E4xF15, were used to map genes that affect H_2S production. Genetic analysis identified three loci, centred on the *MET2*, *MET5* and *MET10* genes, which are responsible for low H_2S production.

Yeast progeny with a *MET2* allele from M2 (or E4) produced less H_2S during fermentation and normal levels of SO_2 (~36 ppm). The yeast *MET2* gene encodes homoserine *O*-acetyl transferase (HTA), which catalyses the conversion of homoserine to *O*-acetyl homoserine. The single amino acid mutation – *MET2*-R301G in M2 (and E4) – is suspected to increase the activity of HTA and lower H_2S formation. Yeast strains that also carried the *MET5* allele (*MET5*-V288del) from M2 (or E4) reduced H_2S even further.

Yeast strains inheriting the *MET10* allele from E4 produced no detectable H_2S during fermentation, and less methionol, carbon disulfide, methyl thioacetate in finished wine. But these strains also produced high levels of SO_2 (~115 ppm) and grew slowly in synthetic grape media with sulfate as the sulfur source. The yeast *MET10* gene encodes the α-catalytic subunit of sulfite reductase, which reduces sulfite to sulfide. The single amino acid mutation – *MET10*-G687D in E4 is suspected to decrease enzyme activity and eliminate H_2S formation in wine.

The new alleles of *MET2*, *MET5* and *MET10* identified here offer significant advantages for developing improved H_2S-preventing wine yeast strains and could potentially help winemakers to produce high quality aromatic wines.

Mapping Gcn1 on the ribosome

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The environment in which cells grow often changes rapidly. In order to survive cells need to adjust their biochemical response to these threatening changes. Vitally important for all organisms are proteins, essential molecules involved in most biological processes in cells. The constant availability of amino acids is therefore crucial, as they are the building blocks for proteins. Yeast and mammals overcome amino acid limitation by switching on a pathway named **G**eneral **A**mino **A**cid **C**ontrol (**GAAC**), which triggers a decrease in general protein synthesis, while upregulating the transcription of stress response genes, finally leading to the *de novo* synthesis of amino acids. For sensing starvation in yeast, the GAAC requires the kinase Gcn2 and its effector protein Gcn1. Gcn2 phosphorylates the α-subunit of the eukaryotic initiation factor 2 (eIF2α), which ultimately induces the expression of stress response genes. To recognize the uncharged tRNA as an immediate signal for starvation, Gcn1 and Gcn2 need to be in direct contact and associated with the translating ribosome. The current model for sensing amino acid starvation is that uncharged tRNAs enter the ribosomal A-site and that Gcn1 is transferring this signal to Gcn2. This model predicts that Gcn1 binds near the ribosomal A-site. To test this prediction, we chose to map the Gcn1 binding sites on the ribosome. We speculate that if a ribosomal protein is important for Gcn1-ribosome interaction and Gcn1 function, then its removal should impair Gcn2 activation under amino acid starvation, as judged by impaired growth on starvation medium and reduced eIF2α phosphorylation. Several large and small ribosomal proteins were uncovered that are required for fully promoting Gcn2 function, and possible mechanisms will be discussed.

Other: Population Genetics

Advancing analyses of microbial population structure: Quantifying gene-flow and connectivity in New Zealand's *Saccharomyces cerevisiae*

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Currently eukaryotic microbial populations are evaluated as either being structured or homogeneous. Just as for macro-organisms, this is unlikely to be biologically accurate. A better approach is to quantify the degree to which microbial populations are differentiated as well as connected by gene flow, but this is largely unexplored. Here we focus on the model research eukaryote *Saccharomyces cerevisiae* which, along with its commercial applications in the production of wine, beer, bread and other alcoholic beverages, has long been used to investigate aspects of cell biology, molecular biology, genetics and evolutionary theory; however, little is known about its ecology and population biology. Previous research by our group has shown that New Zealand harbours a genetically diverse population of this species. Using a microsatellite genotyping system, here we quantify the degree of both population structure and connectivity of this sexual yeast throughout New Zealand by analysing samples of vineyard soil, spontaneous ferments and native plants collected from Hawkes Bay, Martinborough, Nelson, the Wairau and Awatere Valley's and Central Otago. Between the different samples within each region no differentiation was detected suggesting these populations are ubiquitous within regions. Between regions a complex pattern of population differentiation overshadowed by human mediated gene flow emerges. Directional migration into large wine producing regions is uncovered which is consistent with the movement of grapes around the country by the wine industry. This study is the first to quantify gene flow in a model eukaryote and demonstrates the impact human activities can have on microbial distributions.

Other

Yeasts associated with *Drosophila* in Australian Vineyards

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Drosophila and yeasts have a known mutualistic relationship in a number of different environments (for example cacti and rotting fruits) and may potentially contribute to yeast dispersal and persistence in vineyard environments. This study explores the ecological interaction between yeast and *Drosophila* in Australian vineyards. In this study, *Drosophila* were collected in traps and with nets from two Victorian vineyards (Mt. Langi Ghiran and Yering Station) at varying distances (up to 500 meters) from the winery during the harvest months of March and April 2013. Captured *Drosophila* were identified to species level via morphological methods while the yeasts were isolated on 'walk' agar plates and were identified via DNA sequencing of the ITS rDNA region. Almost 500 *Drosophila* were captured in this study. On average, 130 colony forming units of yeasts were isolated from each *Drosophila*. *D. melanogaster/ simulans* were the most commonly found vineyard species of fly, and were associated with the yeasts *Metschnikowia pulcherrima*, *Pichia membranifaciens*, and *Hanseniaspora valbyensis*. *D. melanogaster/ simulans* were numerous at grape waste piles near wineries, where the yeasts *Hanseniaspora uvarum* and *Torulaspora delbrueckii* were also found. Interestingly, the primary wine fermentation yeast, *Saccharomyces cerevisiae* was not isolated in this study. Further results will be presented based on the yeasts associated with male or female flies, and the geographic distribution of *Drosophila* and associated yeasts at these vineyard sites.

Use of small GC-rich mRNA stem-loops 5' proximal to the AUG start codon predictably tune gene expression in yeast.

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There is a lack of tools for precisely tuning gene expression in yeast. Tuning expression levels is critical for reducing the metabolic burden of over-expressed proteins, limiting the accumulation of toxic intermediates, and for reducing or redirecting metabolic flux from native pathways into novel engineered pathways without affecting the viability of the host. We have developed a yeast membrane protein hyper-expression system with critical advantages over conventional, plasmid-based, expression systems. However, expression levels are sometimes so high that they adversely affect protein targeting/folding or the growth and/or phenotype of the host. The objective of this study was to use small synthetic mRNA control modules to predictably tune protein expression levels to any desired level. Down-regulation of expression was achieved by engineering small GC-rich mRNA stem-loops into the 5' UTR which inhibited the translation initiation of the yeast ribosomal 43S preinitiation complex (PIC). Exploiting the fact that the yeast 43S PIC has great difficulty scanning through GC-rich mRNA stem-loops, we created yeast strains containing 17 different RNA stem-loop modules in the 5' UTR and found that they expressed varying amounts of the fungal multidrug efflux pump Cdr1p from *Candida albicans*. Increasing the length of mRNA stem-loops that contained only GC-pairs near the AUG start-codon led to a ~2.7-fold decrease in Cdr1p expression for every additional GC-pair added to the stem, while the mRNA levels were slightly increased. An mRNA stem-loop of only seven GC-pairs reduced Cdr1p expression levels by >99%, and even the smallest possible stem-loop of only three GC-pairs inhibited Cdr1p expression by ~50%. This simple cloning strategy to fine-tune protein expression in yeast has many potential applications in metabolic engineering and the optimization of protein expression in yeast.

Optimising yeast bioethanol fermentation

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Saccharomyces cerevisiae has high stress tolerance and is widely used in bioethanol production. Our approach to improving the efficiency of bioethanol fermentations is via enhancing ethanol tolerance and thereby ethanol productivity through appropriate supplementation of growth media composition. Previous studies indicated that inositol supplementation increases yeast plasma membrane phosphatidyl inositol (PI) levels. This reportedly leads to improved cell growth, stress tolerance and fermentation performance as well as higher plasma membrane H^+-ATPase activity. On the other hand, excess inositol reportedly impairs growth and fermentation performance. Consequently we seek to elucidate the level of inositol providing optimal enhancement of fermentation performance. To date we have assessed the fermentation performance of three strains of yeast in fermentations in defined medium (YNB) with 5%, 10% or 15% (w/v) glucose, to characterise the conditions where maximal differentiation of responses may be seen. Under these conditions, one yeast strain produced higher biomass levels, which were quite susceptible to the higher glucose concentration. The other strains had lower biomass accumulation which were not affected by increasing glucose levels. Viability of all three strains decreased as fermentation progressed over 7 days, with some strains maintaining better viability, presumably leading to better ethanol conversion. All three strains fully consumed 5% glucose over 36h, however when higher gravity conditions were employed the glucose utilisation varied widely. At 10% glucose, the best strain exhausted glucose by 84h, the second by 144h, with the last becoming stuck at 2% residual sugar. At 15% glucose, strains continued to ferment slowly with residual glucose levels of 5%, 8% and 10% at 168h. While use of rich media has been shown to allow these strains to fully utilise higher levels of glucose, their use in this study is contraindicated as inositol levels are generally too high in the initial medium to allow assessment of zero or low level supplementation. Measurements of ethanol levels are currently being confirmed and finalised. We were concerned that measured ethanol levels were lower than anticipated given the glucose consumption data. Alternative analysis of ethanol via HPLC has revealed that this may be due to significant diversion to glycerol production under the higher gravity conditions. This provides us with data to refine the experimental system to better elucidate effects of inositol supplementation prior to moving to industrially relevant media.

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Direct contact between Gcn1 and small ribosomal protein Rps10 is required for full activation of Gcn2 stress sensing kinase

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The ability of a cell to detect and respond to changing amino acid levels is key to its survival in periods of nutrient starvation. In eukaryotes, amino acid shortage is sensed in the form of uncharged tRNAs by the protein kinase Gcn2, which in turn phosphorylates the α subunit of translation initiation factor 2 (eIF2α), leading to a selective synthesis of stress-responsive proteins. Studies in yeast suggested that this sensing requires the binding of Gcn2 to its effector protein Gcn1 and that both must contact the translating ribosomes. We have proposed that under starvation, uncharged tRNAs occur at the ribosomal Acceptor (A)-site and that Gcn1 is directly involved in transfer of this starvation signal to Gcn2. Supporting this model, here we provide evidence that Gcn1 directly interacts with the small ribosomal protein Rps10, which is located close to the ribosomal A-site. A Gcn1 fragment of amino acids 1060 to 1777 showed a yeast two-hybrid interaction with Rps10A, and this interaction was further confirmed by *in vivo* co-immunoprecipitation assays. In addition, purified Rps10A and the same Gcn1 fragment were shown to interact *in vitro*, in a manner independent of RNA. Rps10B, a paralog that differs from Rps10A by only three amino acids, also co-precipitated Gcn1[1060-1777]. Deletion of either Rps10A or Rps10B rendered cells sensitive to amino acid deprivation, and this was associated with reduced phosphorylation of eIF2α, indicative of impaired Gcn2 activation. Overexpressing Rps10A or Rps10B also caused a growth defect on starvation medium, which was exacerbated by the M7A mutation in Gcn1 known to weaken its affinity to ribosomes. Furthermore, we found additional ribosomal proteins, located near Rps10 on the ribosome, whose overexpression conferred sensitivity to amino acid limitation, and this again correlated with reduced levels of phosphorylated eIF2α. Together, these results suggest that Rps10 provides a critical contact point for Gcn1 near the ribosomal A-site and that this is essential for full activation of Gcn2 upon amino acid depletion, supporting the idea that Gcn1 is directly involved in relaying the starvation signal from the A-site to Gcn2.

Genome wide screening for the identification of genes influencing colour in red wine fermentations.

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A micro-fermentation approach was used to screen the yeast homozygous deletion library (~4,700 genes) to identify genes with the potential to modify the pigmentation of model fermentations of a chemically defined medium. Monitoring of residual sugar identified those genes important for the completion of fermentation. The colour values ΔE, ΔC and ΔH calculated from absorbance measurements were used to determine whether there was a measurable positive or negative effect on colour. A positive effect was determined as an increase in Chroma (C* or degree of colour saturation) and/or increase in hue (resulting in a colour change towards an orange-brown colouration). Conversely, a negative effect was either a calculated decrease in chroma and/or decreased hue (resulting in a pink-purple colouration). Pigmentation of yeast biomass was visually determined from photographic images of the cell pellets at the end of fermentation. The fermentation screen was performed in duplicate on a subset of genes identified in the initial screen: 319 genes (negative effect on L*) and 359 genes (positive effect on L*). These datasets were reduced to 94 genes and 50 genes respectively. Interestingly, 50 out of the 94 genes associated with a negative impact on wine colour were associated with increased coloured pigmentation of the biomass. The presence of only 4 genes (coloured biomass) in the 50 gene dataset (positive colour effect) suggests that increased colour is not due to cellular adsorption. GO analysis of the colour data was not straightforward because of the difficulty in finding any meaningful gene associations, for example anthocyanin degradation and metabolism and cell wall synthesis. Whilst many of the genes identified have no known function, of the remainder no gene groups which might be considered obviously linked to the observed phenotype were identified by our preliminary analysis. Laboratory (100 mL) scale fermentation trials will be conducted on the yeast deletants to determine the impact of these gene deletions on sugar catabolism as well as colour. The yeast processes responsible for increased wine colour remains difficult to determine, however, the effect on wine decolourisation is a more attainable goal.

Improved utilisation of alternate nitrogen utilisation by winemaking yeast during fermentation

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Fermentation is a crucial step in wine making. During this process, deficiency of assimilable nitrogen for *Saccharomyces cerevisiae* can cause stuck or sluggish fermentations. Proline represents a significant source of nitrogen in grapes, but is largely inaccessible to yeast during fermentation. Proline utilisation by *S. cerevisiae* only occurs when preferred nitrogen sources are no longer available and requires oxygen. Improved utilisation of proline by yeast would alleviate the need for nitrogen supplementation, benefitting microbial stability and wine quality.

Mutagenesis, involving ethyl methanesulfonate (EMS), was used for the generation of proline-utilising strains. Two desirable isolates, Q2 and Q7, were obtained by growth on a selective medium containing methylamine (ammonium analogue) and proline. Fermentations were performed in Chemically Defined Grape Juice Medium (CDGJM) containing 200g/L sugar, high proline content and low levels of other amino acids and ammonium, under either aerobic or anaerobic conditions. In each case, proline was removed to a greater extent by the Q2 and Q7 isolates, compared to the wild type control. The same trend was also observed during fermentation of Chenin Blanc grape juice fermentation.

Lipid epistasis identifies therapeutic targets to treat Niemann-Pick type C disease

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Niemann-Pick type C (NPC) disease is a fatal, paediatric neurodegenerative and gastrointestinal disease due to lysosomal accumulation of cholesterol and sphingolipids. Currently there is no effective therapy to treat NPC disease. Using the yeast model of NPC disease and genome-wide analyses of conditional synthetic lethality, we identified 18 genes that exacerbated lethality disease severity by manipulating sterols via anaerobiosis (an auxotrophic condition that requires yeast to utilize exogenous sterol) and sphingolipids via myriocin (a pharmacological modifier of sphingolipid homeostasis). Since deletion of histone acetyltransferase genes conferred anaerobic inviability, we used a clinically approved histone deacetylase (HDAC) inhibitor (suberoylanilide hydroxamic acid, SAHA, Vorinostat, Zolinza®) to treat human NPC patient fibroblasts and reverse the major diagnostic criteria of NPC disease (the lysosomal accumulation of both cholesterol and sphingolipids). We will present the translation of Vorinostat and additional unexpected promising targets from yeast to mammalian models of NPC disease in which these targets impact lipid accumulation, life span, and disease markers such as weight loss, ataxia, and Purkinje cell loss. We have thus established an “exacerbate-reverse” approach wherein pathways that exacerbate lethality in a model organism can be reversed in human cells as a novel therapeutic strategy.

Both proline-rich region and actin-binding domain of Las17p are required for high-copy rescue of the *vrp1Δ* temperature-sensitive growth phenotype in *Saccharomyces cerevisiae*

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Introduction / Background / Aim: Las17p and Vrp1p are two interacting yeast proteins that play an important role in the actin cytoskeletal rearrangements that are required for various cellular functions including endocytosis and cytokinesis. In previous work we have demonstrated that yeast cells (*S. cerevisiae*) in which *VRP1* has been deleted (*vrp1Δ*) are viable at 24°C, but not at 37°C. We have also shown that overexpression of Las17p can rescue this temperature-sensitive growth phenotype. The aim of this study is to determine the mechanism by which this occurs.
Methods: Multiple fragments of Las17p were cloned downstream of the *LAS17* promoter and in-frame with a Pk (V5) epitope tag in a yeast high-copy-number (2μ) vector. These constructs were then individually transformed into *vrp1Δ* cells and analysed for their ability to rescue the temperature sensitive growth phenotype. **Results:** Expression of a Pk-tagged N-terminal fragment of Las17p (residues 1-150) known to bind Vrp1p, a large proline rich fragment (151-530) known to bind Src Homology 3 (SH3) domains, or a C-terminal WASP Homology 2 (WH2) domain known to bind actin (531-633) could not individually rescue the growth defect of *vrp1Δ* cells. In contrast, expression of a Pk-tagged fragment containing both the proline-rich region and actin-binding domain could rescue the growth deficiency. Further fragments containing smaller pieces of the proline-rich region and the actin-binding domain have been generated and are currently being evaluated for their ability to rescue the temperature-sensitive growth phenotype.
Conclusion / Clinical Implications: Both the proline-rich and actin-binding domains of Las17p are required for rescue of the temperature-sensitive growth phenotype of *vrp1Δ* cells. Further experiments are aimed at identifying the proteins that interact with the Las17p proline-rich region and the effects of Las17p over-expression on the endocytosis and cytokinesis defects of *vrp1Δ* cells. The human homologues of Las17p (Wiskott-Aldrich Syndrome Protein/WASP) and Vrp1p (WASP-Interacting Protein/WIP) have been implicated in various diseases including immunodeficiency and cancer. Therefore, an increased understanding of the function of these proteins has the potential to impact the treatment of these diseases.

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The mitochondrial ERMES complex in *Candida albicans*

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The importance of pathogenic fungi in the context of human health has increased dramatically over the last few decades. This is primarily due to a rise in the number of people suffering from conditions that affect immune competence. The close evolutionary relationship between fungi and humans is prohibitive for designing safe therapies, and thus very few options for treatment exist. Mitochondrial function has been implicated in antifungal drug susceptibility and virulence of human fungal pathogens. There are several fungal mitochondrial proteins that do not have close homologs in animals, but despite being a potential source of pathogen-specific targets for future antifungal therapies, the function of mitochondria in fungal pathogens is largely understudied. We are studying the mitochondrial ERMES (Endoplasmic Reticulum Mitochondria Encounter Structure) complex in the major human pathogen *Candida albicans*. This complex is widely conserved in fungi, but not present in animals. In *S. cerevisiae*, ERMES has been shown to consists of five subunits (Mmm1, Mdm10, Mdm12, Mdm34 and Gem1), which act to tether the endoplasmic reticulum and mitochondrial membrane systems presumably to enable exchange between the two organelles. We show that in *C. albicans* the ERMES complex impacts on fitness and the susceptibility to the echinocandin cell wall inhibitors. The *C. albicans* ERMES mutants display mitochondrial DNA loss and mitochondrial morphology defects. Interestingly, these mitochondrial phenotypes can be separated from the growth defects of the ERMES mutants. Current studies are aimed at understanding the basis for the essential cellular function of ERMES in *C. albicans*.

Evaluation of fermentation efficiency of yeast *Saccharomyces cerevisiae* *Δtps1* and *Δtps2* deletants with over-expression of *HXK2* and *TreA* genes

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In the yeast *Saccharomyces cerevisiae*, trehalose biosynthesis is mediated by trehalose-6-phosphate synthase (Tps1p) and trehalose-6-phosphate phosphatase (Tps2p). Screening of a commercial yeast deletion library for the ability to complete a high sugar fermentation identified nine gene deletants that resulted in incomplete or 'stuck' fermentation. These nine mutants included *Δtps1* and *Δtps2*. The deletion of *TPS2* has previously been shown to result in the accumulation of trehalose-6-phosphate (Tre6P), which in turn inhibits hexokinase activity, consequently affecting glycolysis. In this study we sought to determine the importance of such inhibition by the intermediate Tre6P by attempting to overcome hexokinase inhibition through overexpression *HXK2* (a paralog of *HXK1* encoding hexokinase isomer 2) in *Δtps1* and *Δtps2* mutants. Over-expression of *HXK2* in *Δtps1* rescued extended fermentation phenotype, producing durations comparable to the control strain BY4743. However, overexpression of *HXK2* was ineffective in *Δtps2*; the mutant was still unable to complete fermentation. In on-going experiments, we are investigating whether glycolysis can similarly be restored by directed degradation of Tre6P via introduction of phosphotrehalase (TreA) from *Bacillus subtilis*. The results from this study will aid efforts to improve yeast strains for successful completion of high sugar industrial fermentations.

Changes in genome organization throughout the cell cycle of *Schizosaccharomyces pombe* correlate with differential gene expression

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The successful progression of a cell through the cell cycle requires the temporal regulation of many biological processes, including gene transcript levels and the number and condensation of chromosomes. Fission yeast (*Schizosaccharomyces pombe*) is a paradigm for cell cycle research and model for higher eukaryotic cells. Due to the size of its genome, it also highly amenable to high resolution studies of the spatial organization of its genome. Here we present the results of a high resolution study in which we used synchronised *S. pombe* cells to investigate cell cycle phase dependent changes in genome organization and transcription patterns. We reveal cell cycle dependent changes in connections within and between chromosomes while confirming previously observed features of genome organization, such as telomere clustering. Our results show that chromosomes are effectively circular throughout the cell cycle and that they remain connected even during the M phase. Determining the structure and transcript levels for matched synchronized cells revealed: 1) that genes with high transcript levels are highly connected with the genome at specific stages of the cell cycle; and 2) that interactions have positive and negative effects on transcript levels. We hypothesize that the observed correlations between transcript levels and the formation and disruption of cell cycle specific chromosomal interactions implicate genome organization in epigenetic inheritance and bookmarking.

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Game theory and the origin of the Crabtree effect

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Social behaviour has long been thought to be relevant for the biology of animals - but not much else beyond. However, in the last 20 years it has been realized that many microbes, including yeasts, display a wide range of social behaviours. They communicate and cooperate, hunt together, fight over food, try to poison each other and have sex. Game theory – the theory behind strategic interaction of “players” in “games” – has been proven to be a valuable tool to analyse such behaviour. In my talk I will discuss how game theory can be used to dissect the evolutionary origin of the Crabtree effect.

Effects of the ribosomal DNA gene repeats on chromosome segregation fidelity

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Cell division is a conserved process involving chromosome segregation, which normally progresses with great accuracy. Despite this accuracy, human chromosomal errors can occur resulting in chromosome gains or losses that are associated with spontaneous abortions, stillbirths, and genetic disorders such as Down syndrome. Human chromosome segregation errors are more frequent in the subset of chromosomes that contain the ribosomal DNA (rDNA) gene repeats. The rDNA genes are essential for cell viability and growth as they encode ribosomal RNA, a main component of ribosomes. Several properties of the rDNA repeats suggest they may affect chromosome segregation. To examine this, we have developed a chromosome loss assay that determines the level of mitotic chromosome missegregation in *Saccharomyces cerevisiae*. This chromosome missegregation assay quantifies chromosome loss by following the fate of selective markers on both sides of the centromere of a single chromosome, chromosome XII, which contains the ~200 rDNA repeat copies. We present evidence for an increase in chromosome missegregation in the absence of rDNA repeats, suggesting that the presence of the rDNA aids faithful chromosome segregation. However, when the wild type number of rDNA copies is reduced, the missegregation rate is higher. One of the consequences of this rDNA copy number reduction is an increase in ribosomal DNA transcription by RNA polymerase I. Likewise, the missegregation rate is high when a key gene, *Fob1*, involved in rDNA recombination and recruitment of chromosome condensation factors to the rDNA locus, is deleted. These results support a role for the rDNA in chromosome separation and suggest that rDNA transcription and rDNA recombination/condensation factor recruitment may be responsible. We are using a genetic approach to unveil the molecular mechanisms behind the role of the rDNA in chromosome segregation. Together, our results reveal a new function for the rDNA repeats in chromosome segregation and show that the rDNA is an interesting system to study the effects of genomic regions on chromosome segregation.

Bimolecular fluorescence complementation (BiFC) studies to investigate protein-protein interactions involved in regulating the protein kinase Gcn2

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Protein-protein interactions are vital to most cellular processes, like regulating the activity of a particular protein, for example. In eukaryotes, protein synthesis is regulated for a rapid response to various types of stress, and this involves several protein-protein interactions. In yeasts, p*hosphorylation of eukaryotic initiation factor-2* α *(eIF2α)* by the protein kinase Gcn2 *(General Control Non-derepressible 2)* is a common regulatory mechanism to adjust protein synthesis in response to various stress conditions including amino acid starvation. Gcn2 is involved in a large array of other seemingly unrelated processes. Gcn2 malfunction has been implicated in many diseases/disorders. This dual nature of Gcn2 suggests that it must be tightly regulated to ensure that it executes the correct function at the correct time and location. So far many proteins *that regulate* Gcn2 have been identified. These act by either directly binding to Gcn2 or interacting with each other. For example, in order to detect its activating signal, Gcn2 must bind to Gcn1. Proteins like Yih1 *(Yeast IMPACT homolog 1)* in yeast and its counterpart IMPACT *(IMPrinted and AnCienT)* in mammals inhibit Gcn2 by occupying the Gcn2-binding site in Gcn1. Yih1 binds actin and inhibits Gcn2 only when dissociated from actin**.** Together with other data obtained in our research group, this strongly suggests that Gcn2 is regulated in a spatiotemporal manner by a dynamic network of protein-protein interactions. The spatiotemporal nature of protein-protein interactions must be studied in their physiological context i.e., in living cells. To accomplish this, we employed a fluorescence-based technique called Bimolecular Fluorescence Complementation *(BiFC)*. Briefly, two non-fluorescent fragments derived from a fluorescent protein are fused to a pair of interacting proteins. When the tagged proteins interact, the two non-fluorescent fragments are brought into close proximity, leading to the autocatalytic formation of the chromophore detectable using fluorescence microscope. Using this technique, we found that Yih1 and actin interact in a specific cellular compartment. These BiFC signals were greatly reduced when extra copies of untagged Yih1 were introduced into the cell, but not when empty plasmid was used, suggesting that the observed BiFC signal was due to Yih1-actin interaction. These findings support our hypothesis that Gcn2 activity is controlled by protein-protein interactions that occur in distinct cellular locations, underscoring the need for further spatiotemporal studies to fully understand how Gcn2 is regulated in the cell.

Investigation of Quantitative Trait Loci Contributing to Drug Response in *Saccharomyces cerevisiae*

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Individuals often undergo a wide variety of responses to drug treatment, in terms of both efficacy and side effects. This variation can arise due to a number of physiological or environmental factors, such as age, disease, diet, and medication error. However, much of the variation also appears to have an individual genetic basis. It has been found that most traits, including drug susceptibility, are not controlled by a single gene, but rather arise from a network of multiple loci, known as quantitative trait loci (QTLs). The budding yeast *Saccharomyces cerevisiae* is a convenient genetic system for investigating drug resistance QTLs in "individuals", as it is a cheap and convenient eukaryote cell model, with a fully-sequenced genome and good homology to human disease genes. Different yeast strains contain similar levels of single nucleotide polymorphism variation as human individuals. Wild type yeast strains can therefore serve as a source of gene sequence variation (which may affect the activity of the gene product) and be used to model differences of these properties between individuals. This work presents an investigation into the genetic basis that underlies yeast cellular response against three widely-used drugs, whose targets are known—atorvastatin (a cholesterol-lowering agent), benomyl (an industrial fungicide), and ketoconazole (a medicinal fungicide)—using a panel of sequenced wild type yeast strains, originally sourced from the Sanger Institute. In my work, this panel of wild type yeast has displayed a spectrum of resistance/sensitivity against each of the three drugs, which was not found to correlate to any mutations or polymorphisms in the gene(s) encoding the primary target for each respective drug. The strains displaying the most extreme drug response phenotypes were mated, and meiotic segregants were tested in terms of their drug response to estimate the number of genetic loci that underlie this phenotype. Finally, we present an approach to use whole-genome sequence analysis to identify the QTLs directly responsible for drug response.

Mapping genes of oenological importance in commercial winemaking yeast

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Deciphering the genetic basis of fermentation traits of the yeast *Saccharomyces cerevisiae* remains a challenge. For this project, two winemaking yeasts were crossed and 96 recombinant F_2 progeny were dissected from the F1 generation. All strains were sequenced using 2nd generation sequencing platforms. 8,200 high-quality sequence variants were identified between the two parental strains. The 96 F_2 progeny were genotyped at these loci and a genetic map of the cross was built. The 96 F_2 progeny were used to ferment Sauvignon blanc juice. Fermentation parameters were modeled using weight loss data. Aroma compounds in finished wines were quantified using SPME-GCMS. Other traits of oenological relevance were measured in additional experiments. One and two-dimensional genome scans revealed quantitative trait loci (QTL) linked to many of the studied traits, including hydrogen sulfide release and the production of volatile compounds. These loci point to candidate genes with mutations between the parents. The relative contribution of a QTL to a trait and its interaction with other QTL were measured and may be useful for breeding purposes. The approach used for this project has proven powerful and accurate for finding genes related to winemaking. The potential of this yeast cross has not yet been fully realized.

4MMP production by *Saccharomyces cerevisiae*

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4MMP (4-mercapto-methyl-pentan-2-one) is an important contributor to white wine aroma with notes of box tree and blackcurrant. It is released by yeast during grape juice fermentation, possibly from cysteine and glutathione conjugates present in the must by the action of Irc7p, a □-lyase enzyme (Roncoroni et al. 2011). It has been shown that strains require the full length *IRC7* gene to release 4MMP.

The aim of this work is to understand 4MMP release by yeast. Our results suggest that low conversion yields of the cys-4MMP precursor to 4MMP are mainly caused by low expression of *IRC7* rather than a low uptake of its cysteine precursor. Known cysteine transporters appear not to be responsible for the uptake of the 4MMP-cysteine precursor, but its transport is diminished by high YAN levels. Overexpression of *IRC7* in yeast confers the ability to grow on L-cysteine as nitrogen source; this characteristic was successfully used to breed high 4MMP producers. Purified Irc7p can cleave L-cysteine to produce H_2S, which has been shown to react with C6 compounds to produce other important varietal thiol (3MH: 3-mercaptohexan-1-ol). The importance of *IRC7* in thiol release will be discussed.

Protein-coding DNA repeats: a major diver of eukaryote proteome evolution?

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Eukaryotic genomes typically contain several thousand genes in which tandem DNA repeats encode amino acid repeats; these can be simple short repeats of the same amino acid or series of repeat motifs each >50 amino acids long. The high number of genes with protein-coding repeats is surprising because DNA tandem repeats mutate orders of magnitude faster than nonrepetitive DNA, potentially generating a stream of inferior protein variants. We have explored possible reasons for the existence of these protein-coding repeats, predominantly in the fungi, using the yeast *Candida albicans* as a starting point. In this species, the existence of repeat alleles specific to different clades, diverged millions of years ago, indicate that many of these genes are used to optimize proteins for a given genetic background, and that novel alleles generated by their mutation may constitute a considerable cost. This is also reflected in the frequent presence of synonymous mutations in many of the protein-coding DNA repeats; these allow retention of the amino acid repeat while reducing, often substantially, the repetitiveness, and thus mutability of the DNA that encodes them. Since synonymous mutations occur much less frequently than deletion of repeat units, the retention of synonymous mutations indicates that selection favours both conservation of the amino acid repeat and reduction of the mutation rate of the encoding DNA. In a survey of 16 eukaryotic genomes, fungal, plant, insect and human, we found that this reduction of mutability is a strikingly consistent feature, as is the distribution of (in silico predictions of) the mutation rates across the protein-coding DNA repeats. We analysed homologues of 17 repeat-containing fungal housekeeping orfs in a variety of eukaryotic species. For each orf we found repeat-free homologues. This indicates that the presence of an amino acid repeat is not indispensible for functionality of these types of proteins. However in slightly over half of fungal homologues a repeat was present in the same location within the orf, although not necessarily encoding the same or even similar amino acid repeats. The most likely explanation of our observation is that eukaryotes incorporate amino acid repeats into their proteins in order to increase rates of proteome evolution to aid in long-term survival in the evolutionary arms race against other species.

Identification of novel Gcn1-binding proteins and their first characterization for their function in Gcn2 regulation

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Gcn2 is an eIF2α protein kinase that was first found in yeast (*Saccharomyces cerevisiae*) to protect cells from amino acid starvation by reprogramming the cellular gene expression profile in response to stress. Now we know that Gcn2 is found in virtually all eukaryotes, and that it protects cells to a large array of stress conditions such as glucose and purine limitation, high salt, reactive oxygen species, viruses, ethanol and UV irradiation. Interestingly, Gcn2 has been found to have acquired additional functions in higher eukaryotes such as cell cycle regulation, viral defence and memory formation. Not surprisingly, Gcn2 has been implicated in diseases and disorders such as abnormal feeding behaviour, Diabetes, Cancer, Alzheimer's, impaired immune response, congestive heart failure, and susceptibility to viruses including HIV. Despite of its medical relevance, so far it is unknown how the cell ensures proper Gcn2 function, and how Gcn2 associated disease can be prevented or rectified. Studies in mammals are hampered by the fact that they contain more eIF2α kinases than just Gcn2. Yeast however only contains the eIF2α kinase Gcn2, making it a superb model organism to decipher in molecular detail Gcn2 regulation. Furthermore, Gcn2 and all Gcn2 regulators found so far are highly conserved from yeast to humans, suggesting that yeast is well suited to understand Gcn2 regulation at the cellular level.

Yeast studies have uncovered that for almost all Gcn2 functions Gcn2 must bind to its positive effector protein Gcn1. Gcn1 is proposed to be a scaffold protein, strongly suggesting that it serves as a platform for recruiting other proteins close to Gcn2 to finetune its activity. For this reason we set out to comprehensively identify all proteins binding to Gcn1, i.e. generate the Gcn1 **interactome**, using a procedure that allowed us to also identify proteins that only weakly or transiently contact Gcn1 (a typical property of regulatory proteins). We found 92 potential Gcn1 binding proteins. Sixty of these were further analysed using the respective deletion strains (sourced from the Yeast *mat a* Deletion Collection). Twelve of these deletion strains were unable to grow under amino acid starvation conditions. Six of these showed reduced eIF2α phosphorylation, strongly suggesting that Gcn2 function is impaired. Using plasmids from the Yeast Genome Tiling Collection, we were able to rescue the Gcn2 function of three deletion strains, indicating that the defect was due to the deletion of the respective gene. So far Kem1 was verified to be required for promoting Gcn2 function. Further studies are under way and an overview of our findings will be presented.

Physical interaction between the Gcn2 regulator Yih1 and the cyclin-dependent kinase Cdc28

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Cells harbour specific control mechanisms that ensure that each step in the cell cycle is completed before the next step commences. The cell surveillance mechanism that delays the G1/S transition acts through the inhibition of the cyclin dependent kinase Cdc28. Cdc28 is a protein kinase that promotes the progression of cells through the cell cycle by associating with different groups of cyclins. It is also involved in the regulation of morphogenesis through the polarization/depolarization of the cortical actin cytoskeleton. Recently, the protein kinase Gcn2 has been found to control the G1/S cell cycle transition upon DNA damage. Interestingly, we have previously shown that the Gcn2 regulator Yih1 binds to Cdc28 (Dautel, Amberg & Sattlegger), and that Yih1 associates with the actin cytoskeleton to promote localized down-regulation of Gcn2, suggesting that Yih1 may provide the link between Gcn2, actin and cell cycle regulation. We are under way characterizing the role of Yih1 in the cell cycle, and will present on overview of our findings.

Does the kinase Gcn2 sense the integrity of the actin cytoskeleton in yeast as found in mammals?

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In virtually all eukaryotes, the protein kinase Gcn2 down-regulates protein synthesis by phosphorylating the alpha-subunit of eukaryotic translation initiation factor-2 (eIF2-alpha) in response to different cellular stresses and starvation. This subsequently leads to the reprogramming of protein synthesis and transcription profile that allow cells to maintain its amino acid homeostasis to cope with stress. Besides these canonical functions, Gcn2 is also involved in cell division, memory formation and cancer progression. Interestingly, we found that the decrease in actin filament levels promoted by treating cells with Latrunculin promptly induces the activation of Gcn2 and the subsequent eIF2-alpha phosphorylation in mouse embryonic fibroblasts cells (MEFs). Gcn2 is activated early after exposure of cells to these drugs. On the other hand, treatment of these cells with the F-actin-stabilizing drug Jasplakinolide has no effect on Gcn2 activation or eIF2-alpha phosphorylation, suggesting that actin microfilament destabilization triggers the activation signal. Moreover, eIF2-alpha phosphorylation is not increased by actin depolymerisation in cells lacking Gcn2, indicating that this is the only eIF2alpha kinase activated upon these treatments. These data suggest a potential novel mechanism of Gcn2 activation in mammalian cells. The actin cytoskeleton network is a dynamic structure that plays important roles in distinct cellular events such as morphology, polarity, movement and endocytosis. In yeast we have previously shown a possible connection between Gcn2 and the actin cytoskeleton in which Yih1 forms a hetero-dimeric complex with G-actin (Sattlegger et al. JBC 279:29952), and when released inhibits Gcn2. These findings seem to suggest that in mammalians, the Yih1 equivalent protein was absorbed by the depolymerised actin to then allow Gcn2 activation. However, considering that the deletion of Yih1 did not lead to Gcn2 activation in yeast, it is plausible to suggest that we may have uncovered a novel mechanism, not involving Yih1, where Gcn2 can sense actin filament disturbance in the cell, possibly as an indicator of the overall cellular well being state. By using both model systems, yeast and mammalian cell culture, we aim to shed more light on this phenomenon and the underlying mechanisms by which actin regulates the protein synthesis through Gcn2.

Investigating the effect of quorum signalling molecules on wine yeast strains and their impact on alcoholic fermentation

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Cell to cell communication between individual cells of a microbial population can lead to co-ordinated behavioural changes in adverse or opportunistic conditions, such that survival of the population as a whole is ensured. While this phenomenon has been studied extensively in bacteria, it has only relatively recently been investigated in yeast. Only a short list of *Saccharomyces cerevisiae* signalling compounds have been identified to date, which includes ammonia and the aromatic alcohols (AroOHs), tryptophol and phenylethanol. Surprisingly the impact of such molecules upon core cellular processes is also largely unknown. Furthermore, information relating to signalling in *S. cerevisiae* is currently limited to laboratory strains and has not been investigated in industrial strains.

In previous studies the filamentous laboratory strain Σ1278b has been shown to form filaments on low nitrogen solid media. This filamentation response has been shown to be enhanced by the addition of AroOHs, either singularly or in combination, to the same media. In this study a number of wine yeast strains, selected for their differing flocculation properties, were evaluated for their ability to form filaments on low nitrogen solid media, both in the presence and absence of AroOHs, compared to Σ1278b. For the first time we have shown that a number of wine yeast strains have enhanced filamentation in response to the addition of these AroOHs. In addition, we observed that there is specific enhancement of invasive growth into solid media, rather than filamentation across the surface of the medium. Interestingly, the amount and type of invasive filamentation across wine yeast strains is quite variable, revealing the potential physiological and genetic diversity between these industrial strains.

Subsequent experiments have investigated the effect of additions of AroOHs to liquid cultures of a selection of these wine yeast strains, in wine like conditions. Cultures were grown to maximum biomass and a small single impulse of the combined AroOHs was added. Fermentation rate, cell growth and cell viability were monitored, however, no differences were observed between cultures with and without AroOH additions. However, quantitative real time PCR, performed on samples taken three hours post AroOH addition, revealed small but significant changes in gene expression of a number of key genes involved in filamentation.

These results show for the first time that industrial *S. cerevisiae* strains are responsive to known yeast signalling molecules on solid media and potentially in liquid cultures. Of particular note is that both nitrogen limitation and the production of AroOHs do occur during industrial fermentations. This suggests that these signalling molecules may be important in the co-ordination of cellular responses of industrial yeast during fermentation.

Controlling protein translation: A message from the 3' end of mRNA

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The control of mRNA translation is one of the many mechanisms utilized by cells to regulate gene expression. How well an mRNA is translated is often determined by 'codes' within the 3' untranslated region (3' UTR). Regulatory molecules, RNA and/or proteins, can bind these codes to influence when, where and how often mRNA is translated. Examples of such regulatory elements are microRNA binding sites, the sites for the Pumillio family of translational regulators, and mRNA localisation factors. However, recent work shows more than 50% of eukaryotic transcripts undergo condition dependent alternative 3' end processing referred to as Alternative Polyadenylation (APA). This generates mRNA isoforms with differing 3' UTR lengths and can result in the gain or loss of the regulatory elements. Variation of the position at which the pre-mRNA 3' UTR is cleaved and polyadenylated can thus provide a mechanism by which the level of protein translation can be graded. That is, a long 3'UTR isoform might contain multiple regulatory elements that silence translation whereas a short isoform may be refractory to such forms of regulation. On the other hand, the steady-state poly(A)-tail length is also controlled by the factors binding the 3' UTR codes. In general, a long poly(A)-tail correlates with active mRNA translation whereas a short poly(A)-tail correlates with mRNA repression, storage and/or decay.

In the laboratory, the translation of mRNA can be conveniently determined by its degree of association with ribosomes. Actively translating mRNA show better ribosomal association than silent, or poorly translating mRNA. In order to explore control of translation, we have established a ribosome affinity isolation assay, which traps mRNA with translating ribosomes. The subsequent comparison between ribosome enriched RNA and the total RNA within the sample, serves as a surrogate read-out of mRNA translation at steady-state. Using this method, our data will demonstrate (1) poly(A)-tail length dependent translational efficiency (2) Condition dependent gene-specific translational regulation (3) the functional consequence of APA. Overall, our studies will provide mechanistic insight into 3' UTR mediated control of mRNA translation.

Halved gene dosage: The impact on alcoholic fermentation

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Several *Saccharomyces cerevisiae* genes have been shown to confer a growth advantage when present as only a single active copy in a diploid individual (*ie.* heterozygous deletion or hemizygous strains). This phenomenon, whereby a halved gene dosage confers an improved phenotype, compared to a wildtype stain, has been termed 'haploproficiency'. Conversely, a hemizygous strain showing a weaker phenotype has been termed 'haploinsufficient'. Our laboratory has been interested in investigating this phenomenon in alcoholic fermentation. A list of candidate genes was formed based on previous findings and prototrophic hemizygous strains of those genes were constructed in a laboratory *S. cerevisiae* background. These were analysed in synthetic grape juice media for a haploproficiency fermentation phenotype. In this study this equated to a reduced fermentation duration compared to the control in micro-fermentations (600 µL) and subsequent laboratory-scale fermentations. Six hemizygotes in the laboratory yeast background completed fermentation approximately 3-10% faster than the control in synthetic grape juice of low nitrogen content. We showed that shorter fermentation duration was associated with increased sugar consumption at earlier time points during fermentation compared to the parent. We sought to determine whether these genes also conferred haploproficiency in a wine strain of *S. cerevisiae*, which might therefore improve fermentation efficiency under industrial conditions. To do this, six hemizygous haploproficient gene candidates were constructed in wine yeast strain L-2056 via homologous recombination of a deletion cassette with one allele of each gene candidate. In contrast to the shorter fermentation duration observed in the hemizygous laboratory strain, hemizygotes in L-2056 showed fermentation duration equal to or greater than L-2056. In a low nitrogen synthetic grape juice, six hemizygotes were observed to have increased fermentation duration (10-20%) while in a high nitrogen synthetic grape juice two hemizygotes were observed to have increased fermentation duration (up to 20%) compared to L-2056. This unexpected haploinsufficiency fermentation phenotype persisted when these hemizygotes were used in fermentations of grape juice. Our results indicate halved gene dosage can both positively and negatively impact fermentation efficiency depending on the yeast strain background. This is likely due to genetic differences between laboratory and wine yeast strains. Also, these findings suggest knowledge gained from studies in laboratory yeast must be further evaluated in wine yeast for industrial application as results may not be directly transferable between genetic backgrounds.

Metabolic Engineering in Yeast Using Systems and Synthetic Biology: Making jet fuel and other fun (bio)chemicals

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Metabolic engineering is the rational re-design of organisms for production of specific industrially-useful biochemicals. In particular, we are interested in producing sustainable, environmentally-friendly replacements for petrochemical products as well as novel industrial biochemicals using cheap, renewable bioprocess feedstocks. The isoprenoid (a.k.a. terpenoid) class of natural products provides many compounds with useful industrial properties, including drugs, plastics, food colourings, fragrances, hormones, and fuel compounds. *Saccharomyces cerevisiae* is a preferred production organism for isoprenoids due to its ability to produce more complex isoprenoids, the relatively high carbon flux through its native isoprenoid pathway, and its relatively good tolerance to solvents used in two-phase bioprocesses. This presentation will focus on novel approaches using systems and synthetic biology to control gene expression and redirect carbon flux for production of isoprenoids and other interesting biochemicals. Examples will include engineering for bio-jet fuel and hijacking the yeast pheromone response system to control gene expression for cell density-dependent production. The former example will focus on systems biology to understand and overcome product toxicity. The latter is a synthetic biology example which achieves dynamic control of engineered metabolic pathways; this is necessary to alleviate the negative effects of both metabolic burden and product toxicity on host organism function.

Overexpression studies – an approach to understand the mechanisms behind successful alcoholic fermentation

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Predictable and reliable alcoholic fermentation by wine yeast *Saccharomyces cerevisiae* at specified temperatures as well as production of desirable flavour and aroma compounds, although not essential to the yeast, are sought-after winemaking attributes. The cellular mechanisms that allow yeast to grow in and respond to the harsh wine fermentation environment and successfully adapt to changing chemical stresses incurred during fermentation are largely unclear. A genome-wide screening approach utilising collections of yeast mutants with individual known gene deletions, is one paradigm being used to address this gap.

Our research has focused on screening collections of yeast with individual gene deletants or gene overexpressers under fermentation conditions, to identify genes that are required for fermentation and which can modulate fermentation outcome. Such genes are representative of what we term the 'fermentome'. We have previously reported on the identification of 93 genes which lead to fermentation protraction upon deletion, a dataset referred to as the Fermentation Essential Genes (FEG).

In this study we report on the construction of an over-expression library in a haploid wine yeast background, which is suitable for fermentation studies in high sugar medium, specifically chemically defined grape juice (CDGJM). This library was screened on a micro-scale (1.8 mL) with the aim of identifying those genes whose over-expression resulted in enhanced or protracted fermentation performance, and lastly, affected the fermentation profile of 36 known yeast-derived aroma compounds. This presentation will focus on preliminary findings on a subset of overexpression strains which are currently being evaluated for fermentation performance at a laboratory (100 mL) scale. Aroma data will not be discussed. It is envisaged that data collated from this study will expand on the FEG dataset as well as other datasets which together, comprise of the yeast 'Fermentome'.

Through collation of gene datasets relevant to fermentation, we are able to identify and understand which genes (and their products) and cellular processes enable yeast to adapt and grow in grape juice and undergo reliable alcoholic fermentation as in winemaking. We have already constructed a number of gene modifications in haploid wine yeast, with the view of producing robust yeast which exhibit robust fermentation under nitrogen limited or high sugar containing CDGJM under laboratory scale conditions. We are also using the knowledge gained from our research to guide selection strategies in directed evolution approaches to generate new 'industry ready' wine yeast with improved fermentation attributes.

Defining the role of the ribosomal DNA in meiotic chromosome missegregation

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Aneuploidy resulting from chromosome missegregation is the leading known cause of congenital birth defects and miscarriage. Most aneuploidies are due to errors in maternal meiosis. Increasing maternal age is a contributing factor due to protracted meiotic prophase I arrest. Interestingly, the acrocentric chromosomes containing the ribosomal DNA (rDNA) tandem repeat arrays are overrepresented in human aneuploidies. We hypothesise that the rDNA repeats may be affecting meiotic chromosome segregation. The model organism *Saccharomyces cerevisiae* can be maintained as both diploid and haploids forms, lending itself to the study of meiosis because all four products of meiosis can be recovered. Because the basic structure of the ribosome, ribosomal DNA regulation, and mechanisms of rDNA transcription and replication are conserved throughout eukaryotes, we utilized *S. cerevisiae* to investigate the effects of the rDNA on meiotic chromosome segregation. Here we present a chromosome missegregation screening system that we are optimising to characterize the effects of the rDNA on meiotic missegregation in *S. cerevisiae*. In this assay, homologous chromosomes in diploid strains are individually marked with separate auxotrophic markers at paired locations. This enables detection of missegregation events in haploid meiotic spores that are isolated using classical methods of spore purification and exclusion of unsporulated diploid cells. We are testing strains with altered numbers of rDNA repeats and defects in genetic pathways that affect rDNA transcription and recombination to further define how the rDNA influences meiotic chromosome missegregation. Comparison with results from mitotic chromosome missegregation assays being undertaken in parallel will enable us to determine the differential effects of the rDNA on meiotic and mitotic divisions.

Investigation and characterisation of an High Nitrogen Efficient (HNE) wine yeast strain

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Sufficient yeast assimilable nitrogen (YAN) is an essential requisite for wine yeast to complete alcoholic fermentation. YAN largely consists of alpha-amino acids and ammonium ions. With adequate YAN (as well as other nutrients) in grapes, processes such as protein synthesis, cell growth and proliferation occur, allowing alcoholic fermentation to proceed efficiently. Insufficient YAN may result in sluggish or stuck fermentation and is often coupled with the formation of undesirable aromas, such as hydrogen sulphide, which impact on wine quality. Thus, the employment of highly nitrogen efficient (HNE) wine yeast provides an alternative strategy to facilitate the completion of alcoholic fermentation under limited nitrogen conditions. In this study, a group of HNE candidate strains were investigated during fermentation of a synthetic medium with different levels of YAN supplementation. Compared with the wild type, a strain with a deletion of *ECM33* showed superior fermentation performance (~ 33% quicker) under either sufficient or low nitrogen conditions. Accordingly the Δ*ecm33* strain is defined as HNE strain.

The role of *ECM33* was further investigated using the loss-of-function Δ*ecm33* mutant. Grow on agar plates containing Calcofluor White (CFW) or Congo Red (CR) was limited, suggesting that *Δecm33* possesses a cell wall defect resulting in increased chitin (target of the antifungals CFW and CR). QRT-PCR results showed that a main chitin synthesis gene, *CHS3*, was upregulated in Δ*ecm33* during fermentation compared with the wild type. The transcriptional abundance of *SLT2* and *HOG1* encoding MAP kinases were also highly upregulated in this mutant. These two kinases have been reported to function in the cell wall integrity (CWI) and high osmolarity glycerol (HOG) pathways, respectively. Based on these observations, we propose that the HNE phenotype of Δ*ecm33* might be triggered by activation of the CWI and HOG pathways, resulting in a strengthened cell wall due to increased chitin content. Such characteristics of *Δecm33* might help the strain adapt to the dynamic environment of the alcoholic fermentation.

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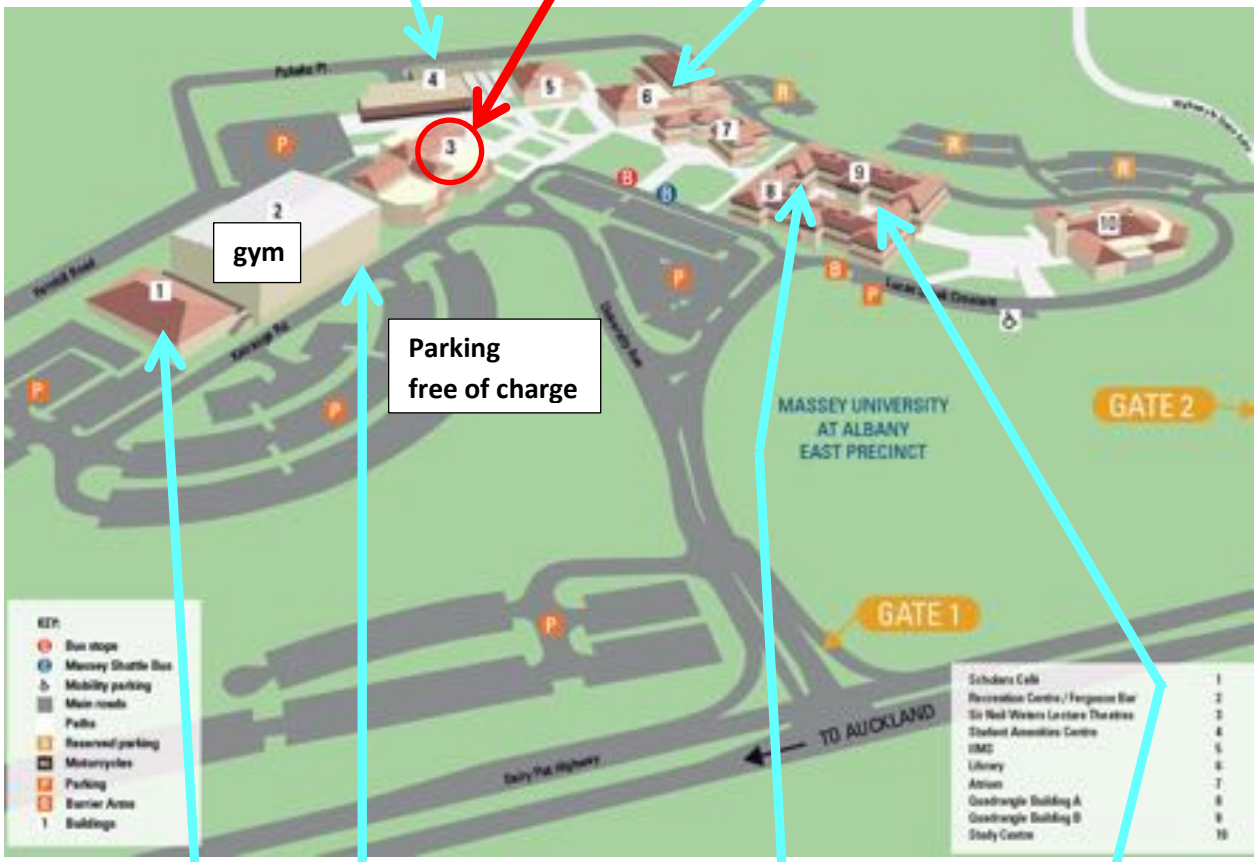
Map, Massey University, Albany Campus, East Precinct

Conference Venue,
SNW Lecture Theatres

Student Amenities Centre:
Dairy & **Pharmacy**, Monday 9am – 5pm
Food for Thought Café, Monday 9am – 3pm
Orbit travel agency, Monday 8am – 5pm
Health & Counselling Centre (Medical centre)
(2nd level) Monday 8:30 – 5pm

Library
Weekends 10am – 6pm
Monday 8am – 10pm
Coffee shop, in Library
Weekends 10am – 6pm
Monday 8am – 6pm



Scholars Cafe
Monday 7am – 4pm

The Ferguson (Bar)
Monday 12pm - late

Quadrangle building

Campus Kebab
Monday 9:30 – 4:30pm

Sushi
Monday 10:30am – 2:30pm

Useful information:

Food
Medical help

Massey University, Albany campus