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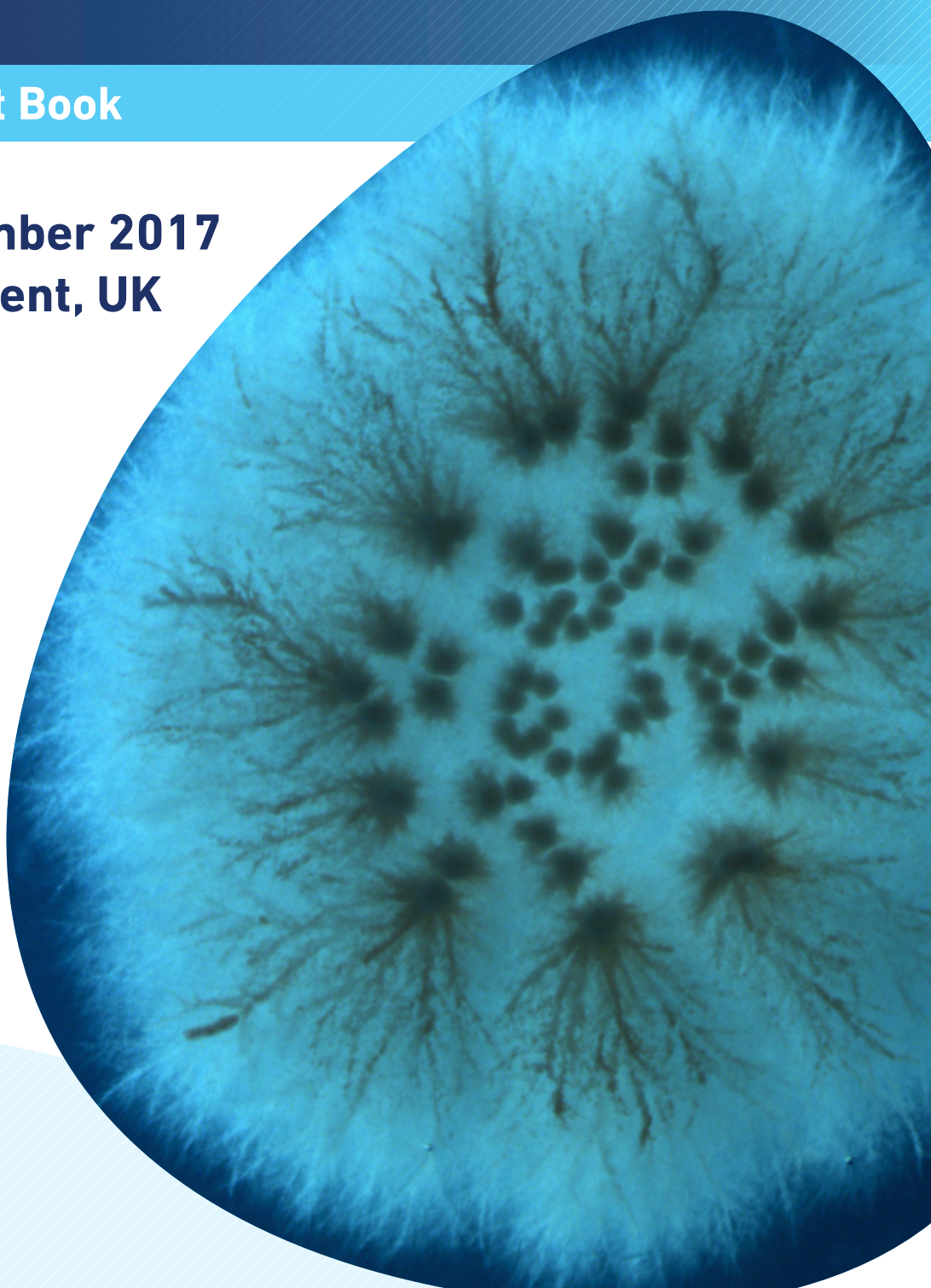

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Poster Number: P01

Elucidating the role of the AP-2 endocytic adaptor complex in *Candida albicans* morphology and virulence.

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Abstract

Fungi such as *Candida* species are a major cause of hospital-acquired infections especially in immuno-compromised patients, and invasive candidiasis is associated with a high mortality rate. Central to *Candida albicans* virulence is its ability to switch between budding (yeast) and filamentous (hyphal) growth, and to colonise different body niches. In each distinct environment it must remodel its cell surface to ensure appropriate levels of transporters and cell wall synthesis enzymes. Endocytosis is known to be a critical pathway in surface remodelling, allowing cells to internalise proteins that are no longer needed at the plasma membrane. Endocytosis is also crucial to highly polarised hyphal growth, where endocytic recycling of key membrane proteins is essential to maintain their polarised location at the hyphal tip.

The aim of this study is to investigate the role of the AP-2 endocytic adaptor complex in endocytosis within *C.albicans*. Homozygous deletions were generated in an essential subunit of the AP-2 complex. The deletion did not affect rates of cell growth but using fluorescence and electron microscopy, defects were observed in hyphal polarization and in cell wall organization. Current studies aim to determine how the AP-2 complex is activated to interact with specific cargoes, and why this is important in *C.albicans* hyphal growth.

Poster Number: P02

In vitro and *in vivo* seeding and cross-seeding of Sup35NM amyloid.

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Abstract

Amyloid seeds are protein particles that are able to accelerate the propagation and transmission of the amyloid protein conformation in a wide range of protein misfolding and transmissible prion diseases. However, the current model of seeded amyloid growth based on templated addition and conformation change of monomers at fibril ends cannot explain why the aggregation of one type of amyloid may exist together with, or synergistically accelerate by the aggregation of another non-homologous amyloid-forming sequences. Here, we address the amyloid cross-seeding mechanism by introducing a model consisting of a combination of templated elongation and surface catalyzed nucleation. Employing a set of *in vitro* and *in vivo* approaches, we experimentally validated our cross-seeding model by studying how seeds of human amyloid-β peptide ($A\beta_{42}$) and yeast Sup35NM accelerate the amyloid assembly of each other. Here, we show that the presence of heterologous amyloid seeds can facilitate amyloid forming reaction for both, $A\beta_{42}$ and Sup35NM despite that they do not normally co-exist and do not share sequence and structural homology. The morphology and the *in vivo* [PSI^{+}] prion phenotype associated with newly formed Sup35NM fibrils are also undisguisable for amyloid fibrils resulting from self-seeded reactions compared with cross-seeded reactions with $A\beta_{42}$ seeds. These data demonstrate that amyloid particles are generic, broad-spectrum seeds capable of accelerating amyloid formation and propagating the amyloid state through surface catalyzed processes. Our results rationalize many unexplained phenomenon observed for diseases-associated amyloid formation, and highlight abhorrent surfaces presented by amyloid particles as a key target for therapeutic intervention.

Poster Number: P03

The Yeast Mep2 Ammonium Transceptor Physically Interacts With The 14-3-3 Protein Bmh1

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Abstract

In response to limiting nitrogen levels diploid yeast undergo a dimorphic switch from yeast like growth to pseudohyphal growth. During this morphological change yeast grow as elongated chains of cells away from the colony to search for nutrients. Studies by a number of groups over many years have established that signal transduction pathways that regulate pseudohyphal growth include the MAP kinase and PKA pathways. An essential but poorly understood component of the regulation of pseudohyphal growth is the Mep2 ammonium importer. Two models of Mep2 function during pseudohyphal growth have been proposed. First, the Mep2 substrate (either ammonium ion, ammonia gas or ammonia gas plus proton) cause changes in cytosolic pH that is sensed by a relevant signal transduction pathway. Second, Mep2 acts as a transceptor that physically interacts with a signalling partner to control pseudohyphal growth. In the transceptor model, Mep2 acts in a way analogous to G protein-coupled receptors undergoing a conformational change during substrate translocation that initiates signalling. We have undertaken a genetic screen to identify potential Mep2 signalling partners and have identified an interaction between Mep2 and the 14-3-3 protein Bmh1. We have confirmed this interaction using western analysis of membrane fractions and demonstrated that this interaction is lost when analysing signalling deficient Mep2 mutants. Importantly, we have identified the 14-3-3 protein binding site in Mep2 which is required for the Mep2 dependent activation of the MAP Kinase pathway.

Poster Number: P04

Engineering mini-chromosomes to study the importance of boundary elements for pericentromere function

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Abstract

Chromosomes need to be partitioned into daughter cells during cellular division. Failure to do so leads to aneuploidy. Cohesin, a ring-shaped protein complex, holds sister chromatids together to ensure their accurate segregation. In *Saccharomyces cerevisiae* a pool of cohesin is loaded at centromeres and spreads out in the nearby pericentromeric region in an unknown mechanism that is thought to involve transcription. Cohesin-enrichment at the pericentromere helps to ensure that duplicated pairs of chromosomes attach to microtubules from opposite poles in preparation for their segregation. The *S. cerevisiae* centromere is only ~125bp, while the cohesin-rich pericentromere is thought to range between 20-50kb in *S. cerevisiae* but is not well defined. Recently, we mapped the pericentromere and identified convergent genes that are enriched with cohesin at the boundaries of the pericentromere on all 16 chromosomes. However the functional importance of the convergent genes at the boundary regions remains unclear.

We have developed a synthetic biology approach to engineer mini-chromosomes that will allow us to study pericentromere establishment and function. To investigate the importance of transcription in establishing the cohesin-rich domain, we generated synthetic pericentromeric mini-chromosomes designed to lack transcriptional units. These presumed transcriptionally inert mini-chromosomes separate precociously, suggesting that cohesion is defective. Ongoing experiments are aimed at introducing convergent gene-pairs onto our synthetic mini-chromosomes to determine if they are sufficient for boundary function and cohesion. Our design, test and analysis cycle will allow us to determine the minimal requirements for pericentromere function during mitosis and the overall translocation mechanism of cohesin across pericentromeres.

Poster Number: P05

Assessing biodiversity of *Saccharomyces sensu stricto* species at different altitudes using culturing methods and metagenomic approach

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Abstract

Natural species of the group *Saccharomyces sensu stricto* are commonly found inhabiting trees bark and surrounding soil, specifically oak trees. Previous attempts to study the diversity of these species in nature involved the use of enrichment culturing methods. In this lab, oak trees bark and soil were enriched using different sugars and incubated at different temperatures to isolate a variety of '*sensu stricto*' species. *Saccharomyces paradoxus* was isolated from soil and bark at 30 °C and 10 °C and *S. kudriavzevii* was isolated from bark samples enriched in melezitose incubate at 10 °C . To avoid culturing bias, mycobiome of soil surrounding oak, spruce and beech trees at altitudes 600m and 1400m were sequenced using Illumina Miseq. The ITS1-5.8s-ITS2 region is typically 850bp in '*sensu stricto*' species, to increase the selectivity this region was extracted and ITS1 was sequenced . Using this approach we were able to detect *S. mikatae*, which was never before isolated in Europe. The metagenomics approach also revealed distinct fungal populations for the different type of tree at the two altitudes. However, compared to other fungal species, a low number of *Saccharomyces sensu stricto* species were detected in general. Our results show coupling culturing method and high-through put sequencing improve the detection of *Saccharomyces* '*sensu stricto*' species in nature.

Poster Number: P06

Quantitative analysis of the operation and control of yeast oxidative protein folding

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Abstract

The production of recombinant proteins with the appropriate post-translational modifications, e.g. oxidation and glycosylation, is imperative for their structure, function and stability, parameters which are important for proteins synthesised as biologics or tools for basic research. Oxidative protein folding, the formation of disulfide bonds, is a key component of a cell's secretory pathway and is dependent on the enzymes Protein Disulfide Isomerase (PDI) and Endoplasmic Reticulum Oxidase (Ero1) for the *de novo* formation of disulfide bonds and subsequent isomerisation to native protein conformation. Whilst yeast-based expression systems are widely and successfully used, their secretory capacity is significantly lower than that of mammalian systems. This work shows the production of yeast PDI and Ero1 orthologues using bacterial host systems and redox modified refolding during purification. This has enabled a quantitative kinetic analysis of their interaction *in vitro*, through measurement of Ero1 dependent oxygen consumption. Oxygen consumption measurements with GSH and reduced and denatured substrate (RNAse A) have highlighted mechanistic information regarding the process of oxidative folding. Computational modelling of this *in vitro* data has permitted the formation of a model capable of predicting the consumption of oxygen, and therefore the total oxidative folding capacity, at different protein concentrations. This quantitative data will enable the rational engineering of yeast strains, and in conjunction with the incorporation of mammalian proteins, such as GPx7/8, will enable the production of super-secreting yeast cells. Such engineering of a 'humanised endoplasmic reticulum' will therefore impact on the future production by yeast of biologics and proteins in general.

Poster Number: P07

Poly-P Mobilisation in the Pathobiology of *Candida albicans*

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Abstract

Candida albicans is an opportunistic fungal pathogen of humans which is able to survive host imposed stresses and successfully proliferate in virtually every anatomical niche within the host. Key to this survival is the ability of *C. albicans* to effectively assimilate essential nutrients, such as phosphate, from diverse host microenvironments. Our recent findings in *C. albicans* revealed that phosphate acquisition, mediated by the Pho4 transcription factor, is essential for growth under phosphate limiting conditions, stress resistance, and virulence of this fungal pathogen. An initial response to phosphate starvation is the release of phosphate from intracellular polyphosphate (polyP) stores within the vacuole. In yeast, the synthesis of polyP and transport to the vacuole is mediated by the VTC complex, and the mobilization of polyP by the polyphosphatases Ppn1 and Ppx1. Here we explored the role of phosphate mobilisation from polyP in regulating phosphate acquisition and stress resistance in *C. albicans*. To facilitate this, strains lacking either the Ppx1 or Ppn1 polyphosphatases were created. However, subsequent analysis of polyP levels within such mutants indicated functional redundancy between these genes. Thus a double *ppx1D/ppn1D* strain has been created allowing for an extensive analysis of the importance of polyP mobilisation in *C. albicans* pathobiology. Here I shall discuss recent data revealing the role of polyP mobilisation in a number of processes in *C. albicans* including phosphate acquisition regulation, metal homeostasis and stress resistance and morphogenesis.

Poster Number: P08

Histone Deacetylase 1 Complex Protein Function in the Pathogenic Yeast *Candida albicans*

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Abstract

Histone deacetylase proteins are known to be important regulators of transcription that impact chromatin structure allowing flexible genetic responses to environmental provocation. The yeast *Candida albicans* is problematic for its human host, disproportionately affecting infants, the elderly, and those suffering from chronic viral conditions. Currently, world mortality rates for fungal infections are high and growing resistance to the relatively small pool of available drugs is a challenge. Therefore, there is an immediate and urgent need for development of new antifungal drugs. It has previously been shown that the histone deacetylase Hda1 plays a critical role in the yeast to hyphae transition, a key morphological switch linked to virulence and pathogenicity. Deleting the gene codifying for the *C. albicans* histone deacetylase Hda1 curbs the switch from round yeast to branched hyphae form which otherwise allows the yeast to traverse human tissue. In the model system Saccharomyces cerevisiae, Hda1 physically and genetically interacts with Hda2 and Hda3. Importantly, these two proteins are conserved in fungi but are not found not in humans. They are ideal targets for antifungal drug development. We are using an interdisciplinary approach to unveil the role of Hda2 and Hda3 in *C.albicans* yeast and hyphal cells. Our genome-wide transcriptomic analysis indicates that Hda2 and Hda3 have distinct roles in specific environmental conditions that separately govern anti-fungal drug resistance and hyphae formation. The results of this ongoing analysis will be presented.

Poster Number: P09

Investigating *Candida albicans* Colonisation of Voice Prostheses Following Laryngectomy

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Abstract

A total laryngectomy is a surgical procedure for people with advanced laryngeal cancer which involves the removal of the entire larynx (including the vocal cords). Speech can be obtained using a small silicone valve, called a voice prosthesis, which is inserted between the trachea and oesophagus. Voice prostheses are susceptible to biofilms which, if left to grow, eventually block the valve, causing the voice prosthesis to fail. One of the main microorganism species colonising these voice prostheses, and certainly the primary fungal pathogen, is *Candida albicans*. Here we show that CO_2 plays a significant role in the promotion of *Candida albicans* biofilm growth on voice prostheses.

The level of CO_2 in exhaled breath is approximately 150x that in normal air (~5% compared to 0.03%) which acts as a powerful inducer of the *Candida albicans* yeast-to-hyphae switch. The yeast-to-hyphae switch is crucial for biofilm growth because hyphal-specific adhesins are important for cell attachment. Furthermore, *Candida albicans* cells locked in either yeast or filamentous growth states produce altered biofilms with reduced biomass and cell density. We have conducted a screen of 165 *Candida albicans* mutants each missing a non-essential transcription factor in an attempt to identify the regulatory elements required for biofilm formation on voice prostheses. Our results indicate there is an important role for iron regulation in this *Candida albicans* biofilm formation, with several of the transcription factors identified having a function in iron homeostasis.

Poster Number: P10

Understanding mechanisms of hormesis and their role in Cancer therapy resistance

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Abstract

Hormesis is known to improve cellular fitness and stimulate other beneficial effects yet the mechanisms remain unknown. To discover novel genes and alleles playing roles in hormetic responses, we used a very high-resolution budding-yeast-based quantitative genetic analysis approach. Our system uses multi-parent F12 advanced-intercross line progeny possessing extensive natural genetic and phenotypic variation. Isolates from worldwide locations (West Africa, Wine Europe, North America, Sake, and China) are used to obtain genetic diversity. By using our high-throughput growth phenotyping approach, 192 arrayed segregants were passaged on low doses of doxorubicin or cisplatin containing YPD agar plates for 11 passages. To assess if the hormetic responses were transient or permanent, cells passaged 11 more times on only YPD plates. Before and after low-dose treatments was then performed for 65-hours with higher doses to detect changes in segregant sensitivities to the agents. Growth phenotype data used to perform r/QTL (Quantitave trait loci) analysis to discover loci influencing the hormetic responses. In total, 37 genes were found (by using *Saccromyces genome database, yeastmine* and *humanmine*) within the loci, 28 of which are conserved. 23 of the genes function in the cell cycle and DNA damage response. Uncovering hormesis mechanisms will shed light on poorly understood adaptive cellular responses. This will benefit drug discovery and overcome drug resistance to ultimately develop better treatments and prevention for cancer and other diseases.

Poster Number: P11

Construction of synthetic system in Saccharomyces cerevisiae to study positive feedback in membrane protein domain formation

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Abstract

Positive feedback is a common feature in biological regulation systems as it allows the cell to maximize the speed and effectiveness of a response to a stimuli. Such phenomena has been studied in transcriptional feedback systems where the expression levels of a gene can be high or low, depending on the expression history of the cell. Similarly, in membrane organisation, positive feedback plays an important role in establishing enriched regions of proteins on the plasma membrane depending on the membrane history. To verify this, we utilize tools of synthetic biology to construct a tuneable system, based on the conversion of Phosphotidylinositol-4,5 bis phosphate (PIP2) to Phosphotidylinositol-3,4,5 triphosphate (PIP3) on the plasma membrane. Since, PIP3 is not naturally present in S. cerevisiae we can fine-tune the system to identify the key features. The system was constructed using an open and closed loop versions of the kinase domain of PI3K, which converts PIP2 to PIP3. The expression of both versions of PI3K was controlled using the GEV system and the amounts of PIP2 and PIP3 on the plasma membrane were observed using florescence microscopy. Our results show that by controlling the levels of PI3K expression, we are able to control the amount of PIP3 on the plasma membrane. We also observed differences between the closed and open loop versions of the catalytic domain. Using this experimental data, we will develop a computational model that can improve our understanding of positive feedback in membrane protein domain formation.

Poster Number: P12

Investigating quantitative trait loci contributing to variation in response to cancer chemoprevention agents for colorectal cancer

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Abstract

Colorectal cancer is the third most common cancer worldwide. Cancer chemoprevention is the use of chemical or natural compounds to prevent or delay the development of malignancy. Numerous studies and clinical trials have been conducted to study the effects of chemoprevention agents however, their mechanism of actions are poorly defined and the outcomes have not been reproducible. This variation in response is majorly due to it being a complex trait controlled by multiple quantitative trait loci (QTL). The aim of this study is to identify the QTLs underlying the variation in response to chemopreventive agents implicated to benefit colorectal cancer patients including metformin, aspirin, curcumin and EPA using a *Saccharomyces cerevisiae* genetic screen. Four parental wildtype *Saccharomyces cerevisiae* strains displaying a wide natural genetic variation were inter-crossed to produce 1000s of genetically diverse segregants to model variation seen in human individuals. 192 segregants were selected in this study and robotically pinned on an array and grown in different chemopreventive agents. Yeast growth was monitored using a novel pipeline which automatically generated R/qtl input files. These files were analysed in a R/qtl software and data was shuffled 1000 times to determine a significance threshold. Hits above the threshold value were flagged as significant QTLs responsible for influencing the sensitivity and resistant of yeast growth to the various agents. QTLs obtained include 100s of genes per agent to which human homologs were found. This study will provide novel insights into the agents' mechanism of action and the development of validated biomarkers for chemopreventive response.

Poster Number: P13

Swr1 mediated H2A.Z incorporation is required to designate centromeres for CENP-A assembly

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Abstract

The centromere is the locus on each chromosome that directs the assembly of the kinetochore, the protein machinery responsible for the accurate segregation of chromosomes through mitosis and meiosis. The underlying hallmark of centromeres in most eukaryotes is the presence of specialised nucleosomes in which canonical histone H3 is replaced by the histone H3 variant CENP-A. While it is widely accepted that specific features of centromeric DNA sequences likely ensure their recognition and mediate a programme of events that installs CENP-A in place of histone H3, the molecular mechanisms that specify the sites of centromere assembly and allow their propagation remain poorly understood. Using fission yeast as a model organism, I am interested in understanding how centromere identity is specified and maintained through regulation of the centromeric chromatin state. Using a combination of biochemistry, proteomics and genomics approaches, I have ascertained the effect of a major chromatin remodeler and histone chaperones on the centromeric chromatin state. Our analyses demonstrate that H2A.Z^{Pht1} and the Swr1 complex are associated with CENP-A chromatin. These factors coordinate deposition of CENP-A through the cell cycle, coupled with the eviction of histone H3 from centromeres, and are required for the de novo establishment of CENP-A chromatin. Based on these observations, we propose that the centromere is programmed to the widespread incorporation of H2A.Z^{Pht1} via Swr1, and that H2A.Z^{Pht1} dynamics likely play a crucial role in regulating centromeric chromatin by influencing CENP-A incorporation.

Poster Number: P14

Novel regulators of NAD+ metabolism in *Saccharomyces cerevisiae*

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Abstract

Nicotinamide adenine dinucleotide (NAD) is a coenzyme involved in numerous cellular process including protein modification reactions. Therefore, maintaining NAD+ homeostasis is essential for proper cellular function. In *Saccharomyces cerevisiae,* NAD+ metabolism has been linked to the phosphate-responsive signalling pathway (*PHO* pathway). PHO pathway regulates NAD+ metabolism trough Nicotinamide Ribose (NR) during NaMN depletion. Although interconnecting biosynthetic pathways to regulate intracellular NAD+ levels has been described, mechanisms regulating NAD+ metabolism remain unclear due to its complex dynamic. In this study, we used a genome-scale model of *Saccharomyces cerevisiae* and flux balance analysis (FBA) to identify novel components and regulators of NAD^+ metabolism. We created *in silico* environmental perturbations to identify genes whose deletion cause a different effect in cellular NAD+ levels. In a media lacking tryptophan and nicotinic acid NAD+ predicted production is reduced by 30% without affecting the growth rate. Under this base-lane condition deletions of *GLN1* and *PRO3* decrease NAD+ predicted production whereas the deletion of *LYS12* increase it. When measured experimentally nuclear NAD+ levels were not affected in *GLN1, PRO3,* and *LYS12* mutants. However, we found that *GLN1* and *PRO3* mutations might activate phosphate-response genes under this condition regulating NAD+ metabolism independently of the Sir2p activity. *LYS12* deletion showed an opposite effect in the gene expression profile. These suggest that NAD+ levels might be re-stablished though NR pathway as soon as the cell senses this depletion, thus masking the predictions. Our findings may contribute to the wider understanding of the regulation of NAD^+ metabolism in higher eukaryotes.

Poster Number: P15

Extrachromosomal regulators of phenotypic heterogeneity in *Saccharomyces sensu stricto*

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Abstract

We are exploring the possibility that phenotypic heterogeneity in *Saccharomyce*s species can in part be explained by the presence of prions, distinct conformational variants of proteins, and other extrachromosomal factors. Previous studies with *S. cerevisiae* have shown that more than 20 different proteins have the ability to undergo such self-perpetuating, heritable changes in their conformation and these changes can have significant impact on the host cell. However, we know very little about the existence or function of prions in other members of the *Saccharomyces sensu stricto* group. To establish whether prions and other extrachromosomal genetic elements act as novel epigenetic regulators of phenotype in three different species from this group: *S. bayanus, S. mikatae* and *S. kudriavzevi,* our initial approach has been to inhibit the function of Hsp104 in these species using guanidium (Gdn) salts (e.g. GdnHCl). This leads to the elimination of the majority of prions in *S. cerevisiae* and as Hsp104 is highly conserved between these species, this should generate prion-free versions of these species. In preliminary studies we have shown that GdnHCl and other Gdn salts can also generate respiratory deficient *petite* mutants in these species and this effect has been investigated at both genetic and ultrastructural level in order to establish how Gdn salts induce mitochondrial defects. Since Hsp104 defective mutants do not show an increased frequency of *petite* mutants in *S. cerevisiae*, the target must be a different molecule and our progress towards identifying that molecule will be reported.

Poster Number: P16

Functional profiling and genetic interaction map of non-coding RNAs in yeast

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Abstract

Background: A protein deletion collection in Saccharomyces cerevisiae has been proven to be useful for functional genomics analyses, however little is known about the molecular function of non-coding RNAs (ncRNAs). We have created a collection of ~450 ncRNA knock-out (KO) mutants in MATa that were used for analysis in this study.

Aims: To create a genetic interaction map of ncRNAs in S. cerevisiae by (i) fitness analysis of double KO mutants generated using Synthetic Genetic Array (SGA) and (ii) screening the double KO library in stress conditions.

Methods: Double KO mutants were generated using SGA by crossing a query strain (carrying single gene deletion) with the ncRNA KO collection. Colony sizes of the single and double KO mutants were recorded. Generated negative and positive scores that represented gain or loss of fitness were used to create scatter-plots and genetic network. Fitness analysis is being performed in various conditions.

Results: To date, ~100 query strains have been generated carrying deletions in snRNAs, SUTs, CUTs and tRNAs. Thirty have been used in SGA to generate double mutations. Several gene interactions have been discovered showing either loss or gain in fitness, such as for example ΔSUT193-ΔSUT055 or ΔSNR13-ΔSUT347. Genetic networks for discovered interactions will be presented as well as fitness results of double KOs.

Conclusions: The SGA analysis on a small number of query strains (27) showed a number of positive and negative genetic interactions, with a few being lethal. Phenotypic analysis of double mutants will help us determine the function of ncRNAs.

Poster Number: P17

Exploiting Diversity and Biotechnological Uses of Hybrids

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Abstract

Hybrids among yeast species and strains are common and are known to be important in many industrial situations including various fermentations used to produce alcoholic beverages. Interspecies hybridization does not only increase the genetic variations in natural yeast populations, it is also an important mechanism for the origin of novel lineages and adaptation to new environments. However, hybrids are difficult to work with, since they are sterile - precluding any genetic analysis and removing the ability to use breeding for strain improvement. In this study, we aim to overcome the hybrid sterility by generating allotetraploid (4n) strains, which can undergo meiosis and produce viable diploid (2n) hybrid spores. We created novel types of hybrids containing different mitochondria to be tested for traits of industrial interests. Specifically, we created interspecific hybrids using a cryotolerant (*S. kudriavzevii*) and a thermotolerant (*S. cerevisiae*) yeast strains. Moreover, we also used the newly discovered *Saccharomyces sensu stricto* species '*S. jurei*' to create interspecific hybrids with *S. cerevisiae*. We showed that upon meiosis the spore viability of the engineered *S. cerevisiae*/*S. jurei* tetraploids was 80-90% and of *S. cerevisiae*/*S. kudriavzevii* tetraploids was 80%. The fitness of the progeny with randomly assorted traits is tested in different environments and in the presence of different stressors to select the best performing hybrids. Ultimately, the genome of improved strains will be sequenced and QTLs underpinning specific traits will be uncovered.

Poster Number: P18

Impact of mitochondrial proteome on the evolution of yeast hybrids

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Abstract

Interspecific hybrids of the *Saccharomyces sensu stricto* species inherit a bi-parental genome but retain the mitochondrial DNA of only one parent. In this environment, chimeric protein complexes can be formed and potentially enhance adaptation to certain stresses. However, several cases of cyto-nuclear incompatibility have been reported where protein complexes are related to lethality and cellular dysfunction. In this study, we aim to investigate the role of mitochondria in the evolution of hybrid genomes by exploring how different abundance of mitochondrial-associated proteins influence the composition of the mitochondrial proteome and ultimately the cellular fitness in hybrids of *S. cerevisiae* with *S. uvarum.* To address this, we used the Fluorescence Correlation Spectroscopy (FCS) microscopy approach to first generate a reference set of absolute quantitative data for the low-abundant Fis1p in intraspecific *S. cerevisiae* and *S. uvarum* hybrid strains growing under respiratory and fermentable conditions. Based on preliminary data, we were able to accurately measure absolute concentrations of GFP-labelled Fis1p in the *S. cerevisiae* parental strains under respiratory conditions at 30^{o}C. From the total of 88-110 molecules per cell of Fis1p, about 90% were unbound and found to be free within cytoplasm. To further study protein-protein interaction and global protein abundance in yeast hybrid, we will monitor several mitochondrial-related proteins (i.e. Mdv1p, Dnm1p) to quantify changes in protein dynamics and their consequences at phenotypic levels. This will essentially help us build an accurate model of the protein pathways in yeast hybrids and understand more about biological systems as a whole.

Poster Number: P19

The role of the MAPK Hog1 in regulating neutral lipid accumulation in *Saccharomyces cerevisiae*

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Abstract

The highly conserved mitogen activated protein kinase (MAPK) High-osmolarity glycerol (HOG) pathway plays a vital role in the ability of *Saccharomyces cerevisiae* to respond to stress. Further, there is evidence that the HOG pathway maybe involved in lipid accumulation following nitrogen limitation as cells enter stationary phase.

To determine the role of the HOG pathway, a nile red assay was undertaken to measure neutral lipid accumulation in gene deletions of key proteins in this pathway. Deletion of most components had no effect, however, the *Δhog1* mutant showed a decrease in neutral lipids, suggesting a role in their accumulation.

Hog1p is activated via dual phosphorylation so this was monitored during the lipogenic switch. Western blots analysis showed an increase in phosphorylation, which corresponds with accumulation of neutral lipids. In the HOG pathway, dual phosphorylation of Hog1 is via Pbs2. However, results from the nile red assay showed deletion of Pbs2p had no effect on lipid accumulation suggesting that Pbs2p is not involved.

Hog1 has a number of downstream targets, but none directly related to lipid production. One potential target is Dga1p, an enzyme involved in synthesis of TAGs, which contains 4 potential MAPK phosphorylation sites. Deletion of *DGA1* both individually and in combination with *HOG1*, results in a similar reduction in neutral lipid levels, suggesting a conserved role. Further work is being undertaken to confirm this interaction.

This work indicates a potential role for Hog1 in regulating lipid accumulation, which may have implications for studying lipid related diseases and biotechnological applications.

Poster Number: P20

THE DEVELOPMENT OF PHOTO-ACTIVATED ANTIFUNGAL DYES AGAINST OPPORTUNISTIC FUNGAL INFECTIONS

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UCLan, United Kingdom

Abstract

The rise in opportunistic fungal infections and the treatment of infectious diseases is one of the most challenging problems in medicine due to the emergence of microbial resistance, side effects and spectrum of activity. A possible alternative to traditional antifungal drugs is photodynamic therapy (aPDT), which depends on the activation of a photosensitiser (PS) by a visible light source to produce reactive oxygen species (ROS) and singlet oxygen, which can inactivate and kill fungal cells.

A library of novel photoactivatable compounds, based on acridine dyes, have been characterised to quantify singlet oxygen release following activation by blue light for 10 and 20 minutes. Candidate compounds were then screened, using the EUCAST microbroth dilution method, for antifungal activity against a range of clinically important fungi including *Candida albicans* and *Aspergillus fumigatus*. Determination of the minimum inhibitory concentration (MIC) has identified a number of novel candidate compounds with activity against *C. albicans.* These compounds were further investigated to determine their mechanism of action using a *hog1* and *msn2/4* genomic deletion strain of *S. cerevisiae.* The findings suggest that the *HOG* pathway has a limited role in the cellular response to the compounds, as the decrease in MIC against *hog1* deletion *S. cerevisiae* was limited. On the other hand, the *msn2/4* deletion strains showed a remarkable increase in sensitivity suggesting that the compounds are inhibiting cell growth via oxidative stress.

Ongoing work in this field may help in the development of a new arsenal of antifungal drugs.

Poster Number: P21

The biological consequences associated with defects in protein disulphide isomerase (PDI) in *Saccharomyces cerevisiae*

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Abstract

Protein disulphide isomerase (PDI) oxidises thiol groups of cysteine residues to catalyse disulphide bond formation (DSB) within select proteins. The DSBs help to stabilise protein conformation and, in addition, can allow for redox regulation of protein activity. PDI also acts as an isomerase, rearranging non-native DSBs to the correct configuration, again ensuring proper protein folding and activity. Such proteins are typically destined for secretion as they transit through the ER-localised oxidative protein folding (OPF) and secretory pathway. *Saccharomyces cerevisiae* secretes far fewer proteins relative to mammalian cells, and consequently the yeast OPF/secretory pathway is considerably less complex. We are undertaking a detailed study of yeast OPF/secretory pathway to establish how changes to the catalytic activity of PDI affects secretion of homologous and heterologous proteins from the cell. To do so we have created eight different yeast *pdi1* mutants to assess the relative contribution of the two active-sites (CxxC) of PDI. In addition, we have investigated the wider biological consequences associated with these PDI defects. Our studies have revealed significant active site residue-specific impacts on protein secretion, but we also demonstrate how the cell adapts to differing forms and degrees of PDI impairment by regulating the expression of other proteins involved in the OPF pathway, and through changes in the activity and responsiveness of the Unfolded Protein Response (UPR) pathway. These, and other studies that will be reported, are allowing us to better understand how PDI is integrated within cellular networks impacting on protein secretion in *S. cerevisiae.*

Poster Number: P22

Investigating spatial regulation through synthetic physical interactions with the yeast centrosome

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Abstract

The Spindle Pole Body (SPB) or budding yeast centrosome acts as a signalling hub, controlling the activity of proteins which localize there. The exact dynamics of protein regulation by localization to the SPB are not fully understood in important processes such as mitotic exit.

Synthetic Physical Interaction screens make use of a strong interaction between GFP and GBP (GFP Binding Protein) tags to force proteins to physically interact. Using a library of GFP strains, these screens may be performed in a high-throughput manner, allowing over 4000 forced interactions to be screened over a week. Such screens were performed with four structural SPB proteins tagged with GBP, effectively forcing the GFP tagged protein to localize to the SPB, and screening for a slow growth phenotype.

Each of these screens identified around 200 reproducible hits. These included Mitotic Exit Network (MEN) proteins, which are known to be regulated through the SPB, reinforcing and expanding existing forced localisation studies of these proteins. Gene Ontology (GO) analysis also revealed enrichment of the results for proteins involved in membrane processes such as lipid biosynthesis and nuclear pore organisation. Furthermore, the screens revealed a number of hits with uncharacterised proteins whose function is currently unknown.

These results support the view of the SPB as a signalling hub and may indicate interactions between the SPB and the local membrane environment in which it is situated.

Poster Number: P23

Dependency of a tRNA^{Tyr} derived ochre suppressor on tRNA modification and eEF1A levels

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Abstract

In *Saccharomyces cerevisiae*, the tRNA^{Tyr} derived nonsense suppressor *SUP4* is able to decode ochre stop codons due to a G to U exchange at the wobble position, generating an UAA cognate anticodon. Using dual luciferase reporter constructs, we quantified the impact of all known modifications in the anticodon stem loop as well as in the variable region of *SUP4* on nonsense suppressor efficiency. To investigate functional interaction between individual modifications, we created combinations of modification defects and scored epistatic, additive or synergistic negative effects on *SUP4* function. The data obtained are in agreement with different degrees of functional redundancy between specific modifications and indicate a complete loss of suppressor function in the absence of wobble uridine and A37 modification. We further demonstrate that gene dose of the elongation factor eEF1A impacts on *SUP4* function and identify a functional rescue of hypomodified *SUP4* by elevated levels of eEF1A. The rescue ability of higher-than-normal levels of eEF1A extends to other combinations of modification defects previously shown to confer severe growth delay.

Poster Number: P24

Mitochondrial development during brewery yeast handling

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Abstract

Until recently the role of yeast mitochondria in brewing fermentations has been poorly understood. Primarily because mitochondrial function is associated with cellular respiration, which is repressed during fermentation due to a combination of the Crabtree effect and predominantly anaerobic conditions. However, current understanding is that mitochondria are essential for cellular function and fermentation performance, due to a number of important characteristics including their role in nuclear DNA signalling, Acetyl-CoA synthesis and lipid generation. This is supported by the fact that cells lacking in mitochondrial function, termed respiratory deficient or 'petites', do not ferment as well as wild type cells. Fermentations conducted using cultures comprising an abnormal number of petites are typically slow with a poor conversion of sugar to alcohol. These yeast cultures display reduced growth, abnormal flocculation, and poor VDK reduction, while the final product is often characterised by irregular flavour profiles. Generation of the petite phenotype is complex and if a critical number of mitochondria are either defective (Rho-) or absent (^{Rho0}) the individual cell will become respiratory incompetent. Conversely, it can be considered that a certain number of mitochondria should be present and able to function correctly for a cell to function as normal. We aim to determine the effect of process conditions on mitochondria number within cells, with the primary goal of establishing some of the key causes behind petite generation. It is anticipated that by furthering our understanding of the petite mutation it will be possible to reduce or mitigate their impact on brewery fermentations.

Poster Number: P25

Characterising the driving forces behind Saccharomyces cerevisiae diversity in New Zealand.

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Abstract

Background

Whilst established as a model organism in a laboratory context, the ecology and evolution of *S. cerevisiae* in the wild is poorly understood. Here we attempt to characterise the driving forces behind diversity in *S. cerevisiae* associated with viticulture in New Zealand (NZ).

Methods

Genome sequences from 104 strains, sampled from 6 sites across NZ, were analysed alongside published data representative of known *S. cerevisiae* diversity. Raw read data was processed using a custom bioinformatics pipeline. Reads were subject to adapter trimming, alignment to a reference genome, sorting and indexing, before variants were called.

Variants were analysed using vcftools, SNPrelate, and the R package PopGenome to calculate population genetic statistics and perform phylogenetic analyses.

Results

Preliminary results suggest the diversity of *S. cerevisiae* in NZ is comparable to that of the larger wine/European group it originates from. However, sliding window analyses of nucleotide diversity suggest that this is not consistent over the entire genome. Previous data indicates that NZ *S. cerevisiae* is composed of 11 subpopulations connected by various levels of gene flow. We aim to confirm this and further investigate the diversity within these subpopulations.

Conclusion

Due to its geographic isolation, NZ provides a unique environment to study the impact that human-aided dispersal can have on *S. cerevisiae* evolution. Current results indicate that while this dispersal has been the main driver behind *S. cerevisiae* diversity in NZ, evolutionary forces have also contributed.

Poster Number: P26

Variation Within Brewing Yeast Populations

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Abstract

Brewery fermentations are unique as it is common practice to harvest the yeast at the end of fermentation then re-inoculate the culture into fresh wort, a process known as serial repitching. Serial repitching often results in declining yeast quality over time, this is dependent on yeast strain and number of repitchings. Some yeast strains can be reused many times with little apparent effect on product quality. However, other strains are less tolerant to repitching, these populations can display genetic and/or phenotypic drift over time, ultimately influencing the capacity of the population to produce beer within product specifications.

Although brewing yeast cultures are typically monoclonal it is known that microbial populations often exhibit differences between individuals. This may result in a decreased fitness to ferment, causing inconsistency, extended vessel residence times and potentially additional downstream processing. This study investigates aspects of population variability to determine the underlying causes behind changes to brewing yeast populations. This includes the potential for selection during repitching, genetic stability of production strains, and analysis of population heterogeneity. Yeast populations were characterised using a variety of DNA fingerprinting methods, and heterogeneity was assessed through analysis of resistance to key stress factors.

This research will enable a deeper understanding of the scientific basis of yeast population variation under stressful but non-lethal conditions. This project is directly relevant to the brewing industry and others which employ fermentation and/or cultivation of yeast on a large scale impinging on a number of sectors worldwide, including baking, oenology, distilling, and animal/human nutrition.

Poster Number: P27

Establishing a reliable metabolomics methodology for S. cerevisiae

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Abstract

Despite the large number of metabolomics methodologies available for analysing the metabolite profile of S. cerevisiae, little consensus exists regarding which methodology is, or should, be favoured. Different methodologies exist for culturing cells, quenching metabolism, metabolite extraction, metabolite detection and assessment of extraction methods, hence making it difficult to draw parallels between the studies and their often-contradictory findings. We feel that assessing the capability of any given methodology to reflect known metabolic changes should be key in determining its future use for biological interpretation. As such, we present recent work that uses the simple, predictable and independent (of metabolomics findings) metabolic influencers of aerobic respiration, anaerobic respiration and ammonium as sole nitrogen source, to assess the suitability of the currently available sample preparation methodologies. Sample separation and detection via UHPLC-MS/MS was then analysed via MS analysis software, MzMine and Omics analysis software, followed by principal component analysis and OPLS-DA using SIMCA. It is hoped that these experiments will establish a sample preparation methodology which accurately reveals the metabolic states of S. cerevisiae and thus can be utilised to reliably identify metabolic change.

Poster Number: P28

Development of a novel algorithm for the segmentation of Schizosaccharomyces pombe cells in phase microscopy images

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Abstract

Robust image analysis is an important aspect of cell biology studies. The geometric properties of cells provide information as to the affect mutations have on cell growth, meaning that the functions and interactions of proteins can be studied in vivo. This type of analysis is highly subjective in nature and time consuming, leading to smaller sample sizes and inaccurate measurements. Automated algorithms that segment cells remove bias, negate human error, and can rapidly scrutinise large volumes of data. To improve the analysis of Schizosaccharomyces pombe populations imaged under phase microscopy, we used MATLAB to develop a fully automated cell segmentation algorithm. Our algorithm has 98.5% precision with 22.1% recall compared to 0.3% precision and 0.9% recall for PombeX, an existing, fully automated program, on the same dataset. Our algorithm can be further improved by parameter optimisation; using ground truths to analyse segmentation success. The latest results will be presented.

Poster Number: P29

Investigating the role of phosphorylation and calcium play in regulation myosin I activity.

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Abstract

Myosins are a highly-conserved group of motor proteins that exert force against actin in order to undertake diverse cellular functions. Class I myosins interact with cellular membranes and provide force sensing anchor between the actin polymers and membrane organelles. Myosin I motor activity is modulated, in part, by interactions with calmodulin, a calcium dependent protein that acts as a light chain for the motor. This lab has identified a conserved phospho-serine on the fission yeast myosin I, Myo1, that plays a role in determining which of two calmodulins interact with the motor. I have been investigating how this phosphorylation dependent switch in light chains effects motor activity and function of Myo1.

Myo1 is phosphorylated at this residue co-commitent with an increase in intracellular Ca2+ during late G1 phase of the vegetative (mitotic) life cycle and later statges of meiosis. It has been hypothesised this Ca2+ shift plays a role in driving the cycle cell forward by facilitating changes modulating competition between light chain interactions with Myo1 and thereby function. I will present my latest findings from studies fluorescence live cell imaging observing dynamics of Myo1, the light chains, Cam1 and Cam2, and endocytic markers in wild type and myo1 phosphomutant S.pombe strains, and our latest models to explain its cell cycle regulation.

Poster Number: P30

Can hybrids reproduce?

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Abstract

Breeding has been utilised by men for millennia to improve various characteristics in animals and plants. It allows for selection of enhanced individuals, which might be better adapted to harsh conditions or produce bigger crops. Hybridisation of closely related species could generate a wide spectrum of possible trait combinations with better or even new characteristics arising. However most hybrids are sterile and as for example in mules the generation of offspring is precluded by reproductive isolation.

Yeast hybrids are found in different locations around the world in industrial environments as well as in the wild. Well known is the hybrid between S. cerevisiae and S. eubayanus called S. carlsbergensis/pastorianus, used in brewing. Furthermore, hybrids of S. cerevisiae × S. kudriavzevii, as well as a complex hybrid of three species known as S. bayanus, are utilised in wine production.

Fertility of interspecific hybrids can be rescued by genome duplication, allowing chromosomes to create pairs of homologues, enabling completion of meiosis. We show that existing hybrids can be improved through breeding. Fertility of a triploid wine strain has been successfully rescued by rare mating events with haploid strain. Therefore, resulting tetraploid individuals were able to successfully complete meiosis, yielding viable progeny. Quantitative genetic analysis, previously precluded by infertility, has now been performed on this wine hybrid. It will reveal the genetic basis of beneficial features (e.g. heat, ethanol or acetic acid stress resistance), providing opportunity for the isolation of strains with potential for benefiting the fermentation industries.

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