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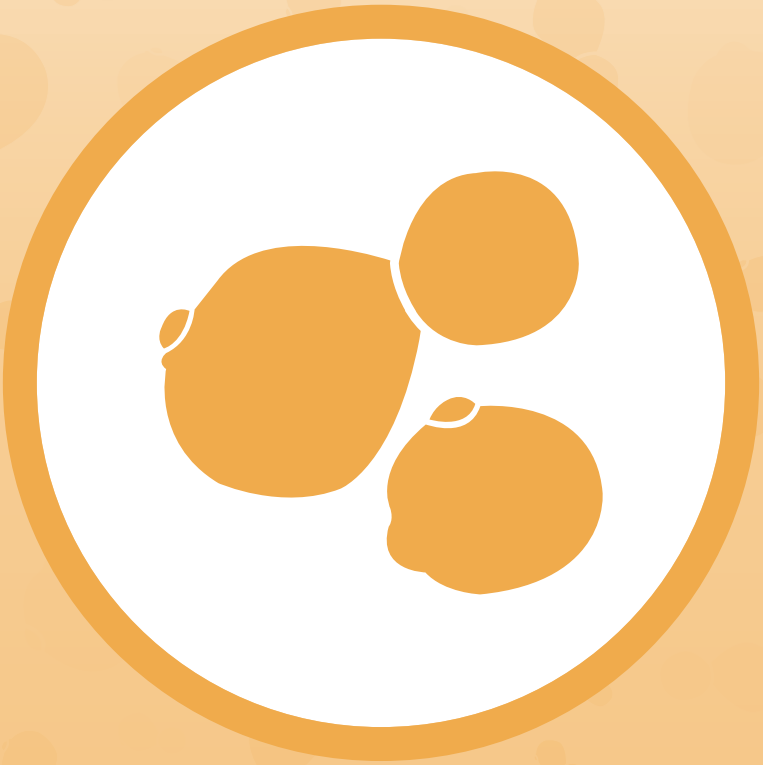

ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

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Identifying the targets of novel compounds using high throughput chemical-genomics in yeast

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To identify the cellular targets of previously uncharacterized bioactive compounds, we employed a chemical genomic (CG) screening platform in *Saccharomyces cerevisiae*, which measured the fitness of mutant yeast strains upon compound exposure. Our CG platform quantifies the relative abundance of hundreds of different pooled yeast strains, in parallel, through a barcode sequencing approach and generates CG profiles that are predictive of compound mode-of-action. We performed comprehensive CG screens using several different sets of yeast mutants, including ~1,000 heterozygous (HET) diploid mutants, ~1,000 temperature-sensitive (TS) haploid mutants and ~1,000 gene overexpression (MoBY) strains, each covering the yeast essential gene set, as well as ~5,000 viable haploid (HAP) deletion mutants, covering the nonessential gene set (manuscript in preparation). We screened these HET/TS/MoBY/HAP strains against a bioactive subset of chemical compounds derived from a collection of ~20,000 molecules in the RIKEN Natural Product Depository (NPDepo). Many compounds had clear CG profiles with specific interactions that may indicate their cellular targets, and comparing the CG profiles derived from each of the four strain collections often provided complementary evidence for target identification. Drug resistant mutant analysis and biochemical assays validated our target predictions. We identified and validated inhibitors for the glycosylation pathway, translation inhibitors which target the phenylalanyl-tRNA synthetase, and inhibitors of the fatty acid synthase.

2

An ultra high-throughput, massively multiplexible, single-cell RNA-seq platform in yeasts that allows for the easy study of hundreds of genetic and environmental backgrounds.

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Yeasts have the extraordinary utility of being naturally diverse, genetically tractable, and easy to grow in a myriad of experimental conditions. Thus, researchers have the ability to investigate any number of genotypes, strains, environments, or the interaction thereof limited only by the processing capabilities of available techniques. Despite their importance to the study of many many biological fields, yeasts have been under-profiled by both RNA sequencing and single-cell RNA sequencing (scRNAseq) methods given current limitations on sample size and number. Here I present a powerful, high-throughput scRNAseq platform that utilizes a combinatorial barcoding strategy to enable massively parallel RNA sequencing of hundreds of yeast genotypes or growth conditions at once. This method can be applied to most species or strains of yeast for a fraction of the cost of traditional scRNAseq approaches. Thus, our technology permits researchers to leverage “the awesome power of yeast” by allowing us to survey the full transcriptome of hundreds of strains and environments in a short period of time, and with no specialized equipment. The key to this method is that sequential barcodes are probabilistically appended to cDNA copies of RNA while the molecules remain trapped inside of each cell. The transcriptome of each cell is labeled with a unique DNA tag without physical isolation, and thus many samples can be combined into a single experiment. In addition to greater multiplexing capabilities, our method also facilitates a deeper investigation of biological heterogeneity given its single-cell nature. For example, in our data, we observe transcriptionally distinct cell states related to cell cycle, growth rate, metabolic strategies, stress responses, etc. just within clonal populations in the same environment. Hence, our technology has two obvious and impactful applications for yeast research: the first is the general study of transcriptional phenotypes across many strains and environments, and the second is investigating cell-to-cell heterogeneity across the entire transcriptome. We are very excited to share it with the yeast community.

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The Rainbow collection – a modern set of yeast libraries for advanced microscopy

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The simple genomic structure of yeast combined with powerful homologous recombination techniques have enabled creation of numerous collections, or libraries, of yeast strains in which each yeast gene is manipulated in a similar manner. Yeast libraries have been available for two decades and have revolutionized the systematic study of cell biology by promoting high-throughput screening of every yeast protein or gene. In particular, the establishment of fluorescent yeast libraries enabled high content analysis at the level of localization and abundance of the entire yeast proteome under multiple genetic and chemical conditions. To date, the utility of these libraries has been hampered by their reliance on basic fluorescent proteins such as the Green Fluorescent Protein (GFP) and the red fluorescent protein mCherry, rendering them unsuitable for modern microscopy techniques. To enable yeast libraries to be compatible with modern microscopy approaches and revolutionize their utility we tested 32 different tags of various spectra, size and purpose enabling us to find nine tags that are efficient and robust for different advanced microscopy techniques. From these nine we have made full genome libraries termed “the Rainbow collection”. Our new collection allows for multiplexing of up to 5 different fluorophores in one screen (using conventional blue, green, red and far-red channels), enables the use of FRET microscopy for the microscopic detection of protein-protein interactions and supports photo-switchable and photoconvertible experiments. In addition, the Rainbow collection includes two libraries of self-labeling tags enabling super-resolution microscopy and biochemical assays. We will demonstrate the power and novelty of each new library and their combinations through investigating key questions in cell biology via high-throughput screening.

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Humanization of the entire sterol biosynthesis pathway in yeast

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Baker's yeast *or Saccharomyces cerevisiae* shares thousands of genes with humans, making them invaluable for studying gene function, genetic interactions, and evolution of critical cellular processes. Humanized yeast, i.e., yeast with human genes functionally replacing their yeast orthologs, offers many advantages, enabling the direct study of human gene function and their disease-causing variants in a simplified model. Systematic functional complementation assays performed one gene at a time revealed "*genetic modularity*" as a primary determinant of replaceability, such that genes belonging to pathways or complexes are either entirely replaceable or not. In the case of non-replaceable genes, we hypothesize that some essential interactions have evolved in a species-specific manner, resulting in incompatibility. Therefore, humanizing the entire processes may restore local genetic or physical interactions, allowing replaceability. We tested this approach to humanize the sterol biosynthesis pathway in its entirety in yeast. While most of the yeast genes in the sterol pathway are humanizable, a pair of two consecutive genes are not. Using a marker-less CRISPR-Cas9 selection and Homology Directed Repair, we show that several human genes can replace their yeast equivalents at their native loci generating single-gene humanized strains. Next, we demonstrate that multiple yeast genes belonging to the sterol biosynthesis pathway are humanizable in a single strain using a sequential approach. Next, we show that a singly non-replaceable yeast gene (*ScERG9/HsFDFT1*) is functionally replaceable only in a partially humanized strain, suggesting that this gene requires a human-specific local genetic or protein-protein interaction context to function in yeast. We also provide evidence that heterologous pathway engineering is cooperative, as shown by the fitness gain (quantitative growth readout) from a lesser to the more humanized system. The resulting humanized yeast strain harbours 8 of 16 human sterol biosynthesis pathway genes. Finally, we design a novel strategy to systematically humanize the remaining genes providing a clear readout of replaceability towards building an entirely human sterol biosynthesis pathway in yeast.

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Profiling of RNA modifications in yeast tRNAs via direct RNA sequencing.

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Decades of biochemical studies have revealed more than fifty unique chemical modifications that decorate eukaryotic tRNAs. These modifications are critical for stabilizing tRNA structure and facilitating de-coding during protein synthesis. Some modifications are conserved at specific positions across tRNAs and across kingdoms of life. Some are subject to dynamic changes during cellular stress. More recently, mass spectrometry and high-throughput sequencing-based approaches have greatly expanded profiling of these modifications, particularly across multiple tRNA molecules simultaneously. We have developed Oxford Nanopore direct RNA-sequencing to sequence all tRNAs, end-to-end, from the budding yeast *S. cerevisiae*. For each condition we measured hundreds of thousands of complete single molecules. Pseudouridine, the most abundant chemical modification on RNA, leads to an altered ionic current disruption as it is pulled through a sequencing pore, as compared to uridine, thus enabling detection of this RNA modification. Our sequencing from mutants lacking pseudouridine synthase enzymes permitted complete assignments of which enzymes catalyze nearly all pseudouridines in tRNAs, matching previous observations while adding estimation of the fraction of each tRNA that is pseudouridylated at each position. Sequencing from cells exposed to chemical and physical stressors, as well as altered nutrient conditions revealed that many pseudouridine sites are robustly maintained across such perturbations, signifying the importance of this chemical modification for tRNA function.

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The genetics of gene expression at single-cell resolution

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Heritable differences in gene expression provide a molecular lens into the biology of traits. Gene expression levels are often measured from bulk populations, obscuring the differences between the effects of expression quantitative trait loci (eQTLs) on single cells due to factors such as their cell-cycle stage. We developed a one-pot approach to map eQTLs in Saccharomyces cerevisiae by single-cell RNA sequencing. We applied our approach to the transcriptomes of over 80,000 single cells of segregants from the extensively studied cross between the yeast strains RM and BY, as well as from two new crosses between strains YJM981 and CBS2888 and strains YPS163 and YJM454. We partitioned these cells into cell-cycle stages using unsupervised clustering in combination with marker genes and mapped tens of thousands of eQTLs, most of which acted independently of the cell-cycle stage. We found 12 new trans-acting eQTL hotspots, and we mapped 11 loci that influence the occupancy of different stages of the cell cycle. In segregants from the cross between YJM981 and CBS2888, we identified a trans-regulatory hotspot on chromosome X that influences the expression of thousands of genes. We fine-mapped the chromosome X hotspot to variants in the gene CYR1, which encodes adenylate cyclase, the enzyme that catalyzes the reaction that produces cyclic AMP (cAMP). This QTL also underlies fitness differences in a wide variety of conditions, and our single-cell data revealed that cells with the CBS2888 allele of CYR1 more frequently occupy the G1 phase of the cell cycle and have increased fitness in 6 stressful conditions. Our results provide a more granular picture of how genetic variants can affect gene expression, cell-cycle progression, and condition-specific fitness.

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Mapping an environmental suppression network of essential genes in yeast

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Genes are typically classified as essential based on the lack of viability of cells deleted for the gene under standard laboratory conditions. However, mutant phenotypes are often modified by environmental factors, suggesting that a proportion of the genes catalogued as essential could possibly be dispensable under different environmental conditions.

To systematically assess the fraction of environment-dependent essential genes, we screened complete deletion alleles of 873 genes (~80% of all essential genes in yeast) for viability in 21 environments targeting a variety of fundamental biological pathways and processes including the maintenance of genome integrity, RNA and protein expression, and osmotic, oxidative and reductive stress response pathways.

Our results suggest that 1 to 10% of essential genes are not required for viability in a specific environment, revealing interesting gene candidates with a strong environment-specific suppression effect. For example, we found that specific glycolytic enzymes were no longer essential in the presence of sorbitol as carbon source, and that several proteins with a role in releasing nuclear export proteins from ribosomal subunits were not required for viability at elevated temperatures. Ultimately, we aim to map a network of high-confidence environmental suppression interactions and to understand the molecular mechanisms underlying differences in gene essentiality and the frequency at which they occur.

8

Sc3.0 : A novel approach to minimize the synthetic yeast genome

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Recent advances in synthetic genomics now allow the synthesis of organisms' entire genomes. The most extensively altered genome built to date is the ongoing Sc2.0 project, aiming to create a synthetic designer *S. cerevisiae*. One of the key features of Sc2.0 project is the insertion of symmetrical loxP sites downstream of every non-essential gene. Upon Cre recombinase induction ***S**ynthetic **C**hromosome **R**earrangement **a**nd **M**odification **b**y **L**oxP-mediated **E**volution* (SCRaMbLE) takes place. SCRaMbLE shuffles the synthetic genome, evolving the synthetic yeast strains toward a desired phenotype by creation of large genotype libraries.

We have shown that SCRaMbLE can minimise the synthetic yeast genome. However, SCRaMbLE cannot delete sequences located within essential rafts, loxPSym flanked units harbouring an essential gene. Essential rafts can move within the genome but cannot be lost due to essentiality restrictions. Supplementing Sc2.0 strain with a Neochromosome housing essential genes would increase the deletion power of SCRaMbLE, facilitating further compaction of the genome. Here, we describe the construction and characterisation of three versions of the essential Neochromosomes harbouring the essential genes of chromosome III (eNeo). The eNeo.v1 contains the essential genes of *S. cerevisiae* with native regulation while, in eNeo.v2 and eNeo.v3, the regulatory elements of the essential genes were refactored to include orthogonal regulatory elements from different yeast species *S.paradoxus* and *S.eubayanus*.

All orthogonal promoters were characterized and the activity of the orthogonal promoters was comparable to the native expression levels of *S. cerevisiae*'s promoter counterparts. In addition, the function of the essential genes driven by orthogonal regulatory prove to be functional and compensate for the absence of the native essential gene in the SynIII strain. Nevertheless, the Random loxPSym-*URA3*-loxPSym integration system was used to select positively for SCRaMbLEd mutants. The deletion of genes was evaluated via PCRtag analysis and other genome rearrangement events were deconvoluted via nanopore sequencing. Introducing the eNeo in the SynIII strain reduces the size of chromosome III effectively, not only by deleting non-essential genes but also by allowing more chromosomal sequences within the essential raft to be eliminated via SCRaMbLE. We anticipate combing SCRaMbLE with eNeo will be an invaluable approach to minimising the synthetic genome.

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Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions

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The Sc2.0 project is building a eukaryotic synthetic genome from scratch, incorporating thousands of designer features. A major milestone has been achieved with all individual Sc2.0 chromosomes assembled. Here, we describe consolidation of multiple synthetic chromosomes using endoreduplication intercrossing. A novel strain with 6.5 synthetic chromosomes was generated and its 3D chromosome organization was evaluated. Its transcriptome was profiled using long-read direct RNA sequencing focused on the effects of designer modifications on transcript isoforms such as loxPsym site insertions and intron deletions. To precisely map "bugs", we developed a method, CRISPR Directed Biallelic *URA3*-assisted Genome Scan, or "CRISPR D-BUGS", exploiting directed mitotic recombination in heterozygous diploids.

Using this method, we first fine-mapped a *synII* defect resulting from two loxPsym sites in the 3' UTR of *SHM1*. This approach was also used to map a combinatorial bug associated with *synIII* and *synX*, revealing a highly unexpected genetic interaction that links transcriptional regulation, inositol metabolism and $tRNA_{Ser}^{CGA}$ abundance. "Starvation" for $tRNA_{Ser}^{CGA}$ leads to insufficient levels of the key positive inositol biosynthesis regulator, *Swi3*, which contains tandem UCG codons. Finally, to further expedite consolidation, we employed a new method, chromosome swapping, to incorporate the largest chromosome (*synIV*), thereby consolidating more than half of the Sc2.0 genome in a single strain.

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Population-level survey of loss-of-function mutations revealed background-dependent fitness variation in yeast

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In natural populations, the same mutation can lead to different phenotypic outcomes due to the genetic variation that exists among individuals. Such genetic background effects are commonly observed, including in the context of many human diseases. However, systematic characterization of these effects at the species level is still lacking to date. The yeast *Saccharomyces cerevisiae* is a uniquely powerful model system to comprehensively survey background-dependent gene essentiality associated with loss-of-function (LoF) mutations. Using a transposon saturation strategy, we recently characterized genome-wide LoF associated fitness variation across 39 genetically diverse natural isolates in *S. cerevisiae*. We found that 15% of genes exhibited a significant gain- or loss-of-fitness phenotype in certain natural isolates compared to the reference strain S288C. Out of these background-dependent fitness genes, a total of 2/3 show a continuous variation across the population while 1/3 are specific to a single genetic background. Genes related to mitochondrial function are significantly overrepresented in the set of genes showing a continuous variation and display a potential functional rewiring with other genes involved in transcription and chromatin remodeling as well as in nuclear-cytoplasmic transport. Such rewiring effects are likely modulated by both the genetic background and the environment. While background-specific cases are rare and span diverse cellular processes, they can be functionally related at the individual level. All background-dependent fitness genes tend to have an intermediate connectivity in the global genetic interaction network and have shown relaxed selection pressure at the population level, highlighting their potential evolutionary characteristics.

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Explicit estimation of mRNA velocity, transcriptional output, and decay from yeast single-cell measurements improves gene regulatory network inference

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Cells respond to environmental or developmental stimuli by changing their transcriptomes through both transcription and regulated mRNA decay. The current state of the art for determining transcriptional rates and RNA decay parameters uses metabolic RNA labeling (e.g. 4-thiouracil pulse-chase) to separate RNA decay from synthesis rates. These experiments are technically challenging and estimates of latent, indirectly measured transcript and decay parameters are not always well-correlated from lab to lab.

We approach this problem by sequencing individual *Saccharomyces cerevisiae* cell transcriptomes by continuously sampling from a population without metabolic labeling. Using this continuous-sampling system, we measure expression in 180,000 individual cells both prior to and in response to rapamycin treatment. Each cell is assigned a position in a temporal space, representing both position on the cell cycle and response to rapamycin. This temporal assignment is based on collection time and comparison to anchoring bulk RNA-seq experiments. The rates of change for each transcript can be calculated on a per-cell basis within the local time neighborhood, resulting in smooth estimates of RNA velocity.

Per-cell RNA velocity is then used to estimate latent, unmeasured transcription factor activities, transcriptional biogenesis rates, and mRNA decay rates as a function of time. We identify transcripts for which transcriptional and RNA decay parameters change in response to rapamycin, and during the progression of the cell cycle. Finally, we use these dynamic (time-dependent) parameters as components of a model to learn the dynamic, organism-scale gene regulatory network that controls yeast.

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Septins interact with AP-3 during vesicle trafficking in budding yeast

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AP-3 is an adaptor protein complex that is structurally and functionally homologous to the AP-1 and AP-2 complexes that sort cargoes into clathrin-coated vesicles which fuse with endosomes. However, AP-3 functions independently of clathrin and sorts cargoes that fuse with the lysosome and lysosome-related organelles. Like AP-1 and AP-2, AP-3 is predicted to function with numerous accessory proteins that facilitate vesicular transport, but few of these accessory proteins have been identified. We searched for protein interactions that drive AP-3 cargo sorting using mass spectrometry to identify proteins that bind AP-3 subunits from lysates of *Saccharomyces cerevisiae*. Among the proteins co-purified were all five septin proteins expressed in mitotically growing yeast. Septins were discovered and named for their function at the plasma membrane during cytokinesis. Bimolecular fluorescence complementation (BiFC) confirmed septins interact with AP-3 in vivo and showed their interaction occurs at discrete puncta frequently located adjacent to vacuoles, which is distinct from septin interactions observed at the plasma membrane. Septin genes with point mutant alleles were also identified in a genetic screen looking for mutants that produce an AP-3 sorting defect, confirming a functional role for septins in the AP-3 pathway. Thus, our studies have identified septins as accessory proteins that function with AP-3.

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Cnm1 mediates nucleus-mitochondria contact site formation in response to phospholipid levels

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Mitochondrial functions are tightly regulated by nuclear activity, requiring extensive communication between these organelles. One way by which organelles can communicate is through contact sites, areas of close apposition held together by tethering molecules. While many contacts have been characterized in yeast, the contact between the nucleus and mitochondria was not previously identified. Using fluorescence and electron microscopy in S. cerevisiae, we demonstrate specific areas of contact between the two organelles. Using a high-throughput screen, we uncover a role for the uncharacterized protein Ybr063c, which we have named Cnm1 (contact nucleus mitochondria 1), as a molecular tether on the nuclear membrane. We show that Cnm1 mediates contact by interacting with Tom70 on mitochondria. Moreover, Cnm1 abundance is regulated by phosphatidylcholine, enabling the coupling of phospholipid homeostasis with contact extent. The discovery of a molecular mechanism that allows mitochondrial crosstalk with the nucleus sets the ground for better understanding of mitochondrial functions in health and disease.

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Ait1 regulates TORC1 signaling and localization in budding yeast

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The target of rapamycin complex I (TORC1) regulates cell growth and metabolism in eukaryotes. Previous studies have shown that nitrogen and amino acid signals activate TORC1 via the highly conserved small GTPases, Gtr1/2 (RagA/C in humans), and the GTPase activating complex SEAC/GATOR. However, it remains unclear if, and how, other proteins/pathways regulate TORC1 in simple eukaryotes like yeast. Here we report that the previously unstudied GPCR-like protein, Ait1, binds to TORC1 in *Saccharomyces cerevisiae* and holds it around the vacuole during log-phase growth. Then, during amino acid starvation, Ait1 inhibits TORC1 via Gtr1/2 using a loop that resembles the RagA/C binding domain in the human protein SLC38A9. Importantly, Ait1 is only found in the *Saccharomycetaceae/codaceae*, two closely related families of yeast that have lost the ancient TORC1 regulators Rheb and TSC1/2. Thus, the TORC1 circuit found in the *Saccharomycetaceae/codaceae*, and likely other simple eukaryotes, has undergone significant rewiring during evolution.

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Isc10 inhibits CMGC group kinases by dual mechanisms

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Many protein kinases activate themselves through the autophosphorylation of their activation loops. Paradoxically, these enzymes must undergo autophosphorylation before they are active. The mechanisms that govern activation loop autophosphorylation remain poorly understood. Smk1, a meiosis-specific MAPK in yeast that regulates spore formation, autophosphorylates its own activation loop upon binding of the activator protein Ssp2. In a previous study, we identified Isc10, a meiosis-specific protein, as an inhibitor of Smk1. Here, we show that Isc10 inhibits Smk1 by two separate mechanisms. First, the C-terminal segment of Isc10, termed the Y Autophosphorylation Inhibitory (YAI) domain, binds to Smk1 and specifically inhibits the *cis*- autophosphorylation of Y209 on its activation loop. In yeast, the YAI motif significantly decreases Smk1 autophosphorylation and spore formation. Second, Smk1 phosphorylates the bound Isc10 on serine 97 (pS97). In turn, pS97 down-regulates Smk1 activity towards *trans*- substrates and other sites of Isc10. Remarkably, Isc10 prevents the mammalian intestinal cell kinase ICK1 (also known as CILK1) from autophosphorylating its activation loop Y, suggesting a conserved mechanism of action. In summary, Isc10 represents a novel class of inhibitory proteins that regulate activation of CMGC group kinases.

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Identification of plasma membrane to trans-Golgi Network vesicle fusion machinery in *Saccharomyces cerevisiae*

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Clathrin-mediated endocytosis (CME) is one of the most important cellular processes for the uptake of nutrients or other cargos at the cell surface. CME is a specialized form of receptor-mediated endocytosis involving clathrin and other endocytic proteins. During CME more than 60 proteins are recruited to plasma membrane (PM) sites for cargo capture, vesicle formation and internalization. Recent studies in budding yeast have shown that yeast have a minimal endomembrane system that is fundamentally different than that of mammalian cells. In this new model, the trans-Golgi Network (TGN), acts as the primary acceptor of endocytic vesicles, sorting cargo for degradation. Given the vast amount of information obtained using the budding yeast endomembrane system, the field must now come to understand these pathways in the context of this new paradigm. Using a targeted genetic approach, we queried SNARE proteins that are known to localize and mediate vesicle fusion events within specific membranes of the yeast endomembrane system. While all yeast SNAREs have been annotated, interestingly none have been identified to mediate the fusion of plasma membrane-derived vesicles to the TGN. Using this targeted genetic approach, we have successfully identified SNARE proteins that mediate PM-TGN vesicle fusion.

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Essential role of the yeast Golgi membrane protein Erd1 in recycling of early Golgi glycosylation enzymes

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Maintenance of the correct subcellular localization of enzymes is critical for cellular homeostasis. Glycosylation is the most abundant and diverse post-translational protein modification mediated by sequential action of transmembrane glycosyltransferase enzymes appropriately maintained in specific Golgi compartments. Using yeast genetics and fluorescence microscopy, we have uncovered the integral membrane protein in yeast, Erd1, as a key facilitator of Golgi glycosyltransferase recycling by directly interacting with both the Golgi enzymes and the cytosolic receptor, Vps74. Loss of Erd1 function results in mislocalization of Golgi enzymes to the vacuole/lysosome. We present evidence that Erd1 forms an integral part of the recycling machinery and ensures productive recycling of several early Golgi enzymes. Our work provides new insights on how the localization of Golgi glycosyltransferases is spatially and temporally regulated, and is finely tuned to the cues of Golgi maturation.

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Leveraging gametogenesis-specific rejuvenation pathways to counteract cellular aging

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At the cellular level, aging manifests as an accumulation of conserved physiological defects that eventually cause functional decline, disease, and organismal death. Surprisingly, the germ line contains inherent rejuvenation pathways that prevent age-associated damage from being passed onto progeny. Therefore, there is strong incentive to understand how gametogenesis-specific genes can eliminate aging biomarkers and determine whether these pathways can be leveraged to counteract cellular aging in somatic cells. Gametogenesis is a highly regulated developmental program whereby diploid progenitor cells undergo cell division (meiosis) and differentiation to produce haploid gametes. Studies in budding yeast have revealed that gametogenesis completely resets lifespan. Our lab has uncovered meiosis-specific mechanisms for removing aging biomarkers; however, the subset of meiotic genes involved in cellular rejuvenation remains largely unknown.

Using mRNA isolated from different meiotic stages, we constructed 5 inducible cDNA libraries to identify meiotic transcripts that can extend lifespan in budding yeast. We developed a screening pipeline to assess the effect of meiotic genes on competitive fitness in both young and old populations of yeast. We identified 80 rejuvenation candidates, which have the potential to become new therapeutic targets for preventing or halting age-associated disease progression. This includes genes with roles in the mitochondria, endoplasmic reticulum, Golgi, vacuole, and RNA processing. The diversity in this list is exciting because it may represent multiple pathways that converge to rejuvenate aging cells. In addition, we find a subset of candidates with unknown, putative, or dubious functions, which could represent understudied meiosis-specific genes or gene isoforms that can provide new unexplored targets for aging intervention. Finally, we find several rejuvenation candidates that are potential targets of the meiotic transcription factor, Ndt80, which may facilitate nucleolar rejuvenation and lifespan extension that has been previously reported. Downstream analysis will be focused on elucidating the rejuvenation mechanisms of these genes.

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Does Rnq1's evolutionarily conserved non-prion domain regulate functional aggregation of Rnq1?

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Prions and prion-like amyloid aggregates are associated with devastating diseases, including Creutzfeldt-Jakob, Alzheimer's, Parkinson's, type 2 diabetes, cancer, Huntington's and ALS. However, accumulating evidence suggests that prion-like aggregates also play an important role in normal cellular physiology, e.g. by driving formation of mRNA processing bodies and localizing protein synthesis to the sites where proteins are used. [*PIN*$^+$] is a prion form of the Rnq1 protein. It promotes formation of other yeast prions. Indeed, the *de novo* formation of the [*PSI*$^+$] prion essentially requires [*PIN*$^+$]. Direct cross-seeding has been proposed to explain this. [*PIN*$^+$] also promotes aggregation of several proteins associated with human neurodegenerative diseases, including huntingtin and TDP43, and inhibits propagation of other prions. The C-terminal prion-forming domain of Rnq1 (PD) contains several Q/N-rich determinants, which can independently maintain [*PIN*$^+$] and promote [*PSI+*] formation. Despite two decades of research, the function of non-prion Rnq1, and the role of its non-prion-forming N-terminal domain (NPD), is still unknown. Using a genetic screen, we found that a mutation in the NPD of Rnq1 inhibits transmission of [*PIN*$^+$] from WT Rnq1 to the mutant Rnq1. However, after rare transmissions, the resulting mutant [*PIN*$^+$] prions can propagate stably. Also, the mutant Rnq1 readily forms new aggregates in [*pin-*] strains. Thus, the mutation creates a transmission barrier: the change in the NPD modulates the conformation attained by the PD within the amyloid aggregate. The distinct appearance of amyloid fibers formed by the mutant vs. WT Rnq1 *in vitro* supports this conclusion. The strength of the barrier varies considerably for constructs containing different QN determinants of the Rnq1 PD. Based on this, on evidence for positive and negative interactions of [*PIN*$^+$] with other prions and amyloids, and on the fact that [*PIN*$^+$] is the only prion found in natural yeast isolates, we hypothesize that Rnq1's cellular function is to regulate aggregation of other proteins. Indeed, *RNQ1* orthologs from other fungal species contain the moderately conserved N-terminal domain (the site of the

above-mentioned mutation is conserved), and C-terminal domains reflect evolutionary amplification of Q/N-rich sequences and have characteristic features of aggregation-prone prion domains.

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Reprogramming single-cell aging in yeast

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Cellular aging is a complex process that involves many interwoven molecular processes. Studies in model organisms have identified many individual genes and factors that have profound effects on lifespan. However, how these genes and factors interact and function collectively to drive the aging process remains unclear. We investigated single-cell aging dynamics throughout the replicative lifespans of *S. cerevisiae*, and found that isogenic cells diverge towards two aging paths, with distinct phenotypic changes and death forms. We identified specific molecular pathways underlying each aging fate and revealed that these pathways resemble a toggle switch that drives the aging fate decision and the progression towards death. Furthermore, based on the knowledge of aging in WT cells, we used synthetic biology approaches to rewire the endogenous network and engineered single-cell aging trajectories toward a substantially extended lifespan. Our results establish a connection between gene network architecture and cellular longevity and set the stage for the rational design of synthetic gene networks that can effectively slow aging.

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Identifying pathways that slow down the age-associated decline of mitochondrial membrane potential

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Mitochondrial function declines as cells age. A major contributor to this decline in function is the decline in mitochondrial membrane potential (MMP). MMP is generated when cells convert metabolic activity into a proton motive force across the inner membrane of the mitochondria. In addition to generating ATP, MMP is required for other essential functions, such as ion and protein import (99% of mitochondrial proteins are encoded by the nucleus), as well as shuttling intermediates of the TCA cycle between the cytoplasm and mitochondria (Kutik et al., 2007, JCB). To better understand the genetic determinants of MMP decline, we utilized a fluorescence-based genetic system that reports MMP-dependent mitochondrial protein import using MMP-dependent and MMP-independent signals. We crossed this reporter into the yeast knock-out library to generate ~4,700 bar-coded reporter strains. The pooled reporter library was aged for 24 hours in miniature aging devices (MAD; Hendrickson et al., 2018, eLife) and sorted by FACS based on the ratio of MMP-independent to MMP-dependent signal (MMP ratio). Barcode sequencing was performed to determine which genes were enriched in the high MMP ratio population. We recovered ~4,600 unique barcodes, and confirmed 57 of the top 69 hits using single-cell microfluidics (Thayer et al., 2022, bioRxiv). In all cases, the hits showed increased MMP when young. The microfluidics data revealed that lifespan increased with initial MMP increased to a point, but in mutants with very high initial MMP, lifespan fell, suggesting there is an optimal MMP that is associated with maximal lifespan. We identified three functionally distinct genes that optimized MMP and lifespan. One of the hits, Sis2, is an inhibitor of the phosphatase Ppz1, suggesting that increased Ppz1 phosphatase activity was responsible for the increase in MMP and lifespan. While Ppz1 is implicated in several important cellular processes, we found that, in a sis2Δ background, deleting the kinases that oppose Ppz1's negative regulation of the K+ transporter Trk1 further increased both MMP and lifespan. Phosphoproteomics revealed that Ppz1 also affects phosphorylation of the mitochondrial import protein SSC1, which may modulate its function. Thus, simultaneous regulation of at least two distinct processes (e.g., pH homeostasis and mitochondrial import) may be necessary to prevent age-associated MMP decline in a way that also increases cellular lifespan.

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Comparative proteomics of the multidrug-resistant human fungal pathogen *Candida auris* links cellular detoxification with amino acid metabolism

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Candida auris is a newly emerging human fungal pathogen, causing hospital outbreaks of disseminated fungal infections in immunocompromized individuals. Echinocandins (Ecan) and amphotericin B (AmB) are the only therapeutic approaches for *Candida auris* as ~30% of clinical isolates are resistant to AmB and ~5% are resistant to Ecan. However, the precise mechanisms underlying complex antifungal-resistance traits in *C. auris* remain enigmatic. We used shotgun proteomics to compare drug-resistant (CauR) with drug-sensitive (CauS) clinical isolates to identify multidrug-resistance mechanisms contributing to complex phenotypes. In brief, clinical isolates were treated or not with AmB before preparing cell-free extracts for LC/MS-MS analysis. By comparing the proteomes of resistant isolates (CauR) with sensitive ones (CauS), we found altered abundance of proteins associated with cellular detoxification, those implicated in amino acid and fatty acid metabolism, as well as proteins required for fungal cell wall architecture and/or drug transport. Remarkably, our data show a high abundance of proteins essential for bicarbonate metabolism, suggesting a critical role for related pathways in antifungal resistance development. Most importantly, we identified a new transcriptional regulator that appears to link antifungal drug resistance with metabolism, oxidative stress and transport-mediated resistance. The altered abundance of this transcriptional regulator was confirmed when proteomics datasets were overlayed with RNA-seq data from these clinical isolates. In summary, the integrative approach of proteomics and RNA-seq is suitable to decipher the molecular basis of pan-antifungal resistance traits in the human fungal pathogen *Candida auris*.

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Mitochondria-dependent toxicity of aggregation prone proteins

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Protein misfolding and aggregation are natural occurrences in living cells. If not properly handled, misfolded proteins can accumulate, cause problems, and lead to disease. For example, aggregation prone proteins are known causes of neurodegenerative disease. When expressed at high levels, such proteins lead to dosage-dependent toxicity in different cells, including yeast. Yeast genetic screens have helped to uncover many conserved genes that modulate the toxicity of these disease-causing proteins. Some modifiers regulate protein homeostasis while others are involved in cellular processes specifically perturbed by toxic protein aggregates. Finding modifier genes provides us with a path to understanding disease mechanisms and developing new therapeutics. In most modifier screens, the *GAL1* promoter is used to express aggregation prone proteins on galactose. One limitation here is that growth on galactose, a fermentable carbon source, does not fully activate mitochondria-dependent cellular respiration. Mitochondria play conserved roles in the control of protein homeostasis in eukaryotes. Mitochondria also have essential functions in neurons. Accumulating evidence supports that mitochondria are targeted by aggregation prone proteins, and mitochondrial dysfunction may contribute to aging and neurodegenerative disease. To include mitochondria as a cellular context for studying the toxicity of disease-causing aggregation prone proteins, we modified the *GAL1* system by introducing a mutant transcription factor to activate gene expression while forcing cells to undergo respiratory growth. Examining yeast cells expressing FUS, TDP-43, Htt103Q, and α-synuclein under both fermentative and respiratory growth, we saw condition-specific toxicity of these proteins. In cells expressing α-synuclein, respiratory growth markedly increases cytoplasmic inclusions and aberrant mitochondria. Both are rescued by a known suppressor, Ypt1. We also found a family of conserved cell signaling proteins showing condition-specific effects when co-expressed with α-synuclein. These proteins severely increase the toxicity on galactose while strongly protecting the cells under respiratory growth. This has allowed us to investigate whether the cell's ability to survive stress induced by α-synuclein is dependent on fully functional mitochondria. Our data support the importance of studying misfolded proteins and their associated cellular toxicity in the context of active mitochondrial function.

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Evolutionary rescue of phosphomannomutase deficiency in yeast models of human disease

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We use experimental evolution to identify mutations that compensate for human-disease associated alleles of the phosphomannomutase gene *SEC53*. Mutations in the human homolog of *SEC53* (*PMM2*) affect protein N-linked glycosylation and are the most common cause of congenital disorders of glycosylation (CDG).

We evolved 384 populations of yeast with either wild-type *SEC53* or one of two human-disease associated alleles. After 1,000 generations, most populations compensate for the slow-growth phenotype associated with the disease alleles. By whole-genome sequencing and genetic reconstruction, we identify both known and previously unknown genetic interactors of *SEC53*. Interestingly, we observe an enrichment of compensatory mutations in genes whose human homologs are associated with other CDG diseases, including *PGM1*, which encodes the minor isoform of phosphoglucomutase. By tetrad dissection and biochemical characterization of purified proteins we show that compensatory *PGM1* alleles are dominant suppressors that reduce, but do not eliminate, enzymatic function.

Disease-associated alleles that impinge upon core and conserved biological processes (e.g. protein glycosylation, ribosome maturation, mitochondrial function, and RNA modification) are likely to be highly pleiotropic. In humans, salient phenotypes may be neurological or developmental, but in yeast, these disease alleles invariably lead to slow growth. We, therefore, propose that experimental evolution, combined with whole-genome sequencing and genetic reconstruction, is a powerful and general strategy for identifying genes and pathways whose modulation can compensate for the deleterious effects of human disease-associated alleles.

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Hybridization drives mitochondrial DNA degeneration and metabolic shift in a species with biparental mitochondrial inheritance

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Mitochondrial DNA (mtDNA) is a cytoplasmic genome that is essential for respiratory metabolism. While uniparental maternal mtDNA inheritance is most common in animals and plants, distinct mtDNA haplotypes can coexist in a state of heteroplasmy, either because of paternal leakage or de novo mutations. MtDNA integrity and the resolution of heteroplasmy have important implications, notably for mitochondrial genetic disorders, genome evolution and speciation. However, knowledge on the factors driving heteroplasmy resolution is sparse. Here, we use *Saccharomyces* yeasts, fungi with constitutive biparental mtDNA inheritance, to investigate the resolution of mtDNA heteroplasmy in a variety of hybrid genotypes. We analyzed the mtDNA sequence and growth phenotypes of 864 yeast lines that were experimentally evolved under relaxed selection for mitochondrial function, allowing us to explore the near-neutral evolution of mtDNAs. The lines are subdivided in 11 different hybrid crosses among natural lineages of *Saccharomyces paradoxus* and its sister

species *Saccharomyces cerevisiae*, yielding a variety of hybrid genotypes along a gradient of parental divergence. We observed extensive mtDNA recombination, but recombination rates were not predicted by parental divergence and were genotype-specific. However, we found a strong positive relationship between parental divergence and the rate of large-scale mtDNA deletions, which lead to the loss of respiratory metabolism. We also uncovered associations between mtDNA recombination, deletion, and genome instability that were genotype-specific. Our results show that hybridization in yeast induces mtDNA degeneration, with deep consequences for mtDNA evolution, metabolism and the emergence of reproductive isolation between species.

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Understanding the likelihood of evolutionary tradeoffs among drug resistant mutations using a large population of barcoded yeast

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Predicting evolution is difficult. One reason for this is that the genetic targets of adaptation are more varied than previously thought. Further, adaptive mutants that seem to behave similarly in their home environment, demonstrate diverse and unpredictable tradeoffs in non-home environments. There is a growing interest in utilizing these evolutionary tradeoffs in evolutionary medicine. For example, collateral sensitivity (CS), where developing resistance to drug A results in sensitivity to drug B, is a type of evolutionary tradeoff that may be exploited to counter drug resistance. While some studies suggest that CS is common, others have demonstrated that CS is unpredictable and nonrepeatable. Previous work has not reached a consensus on the likelihood of evolutionary tradeoffs, like CS, partly because most work utilizes small experiments with low replicate numbers and cannot survey the wide array of mutations that can contribute to drug resistance nor the prevalence and predictability of their tradeoffs.

Here, we use a barcoded *S. cerevisiae* system to track a large population of yeast strains as they develop resistance to different drugs at varying concentrations (drug A; n=12). We tracked 200,000 replicate yeast lineages to reveal many different adaptive mutants that protect against each drug. From these evolved populations, ~30,000 strains were then subsequently challenged by a second drug (drug B; n=12) and mutants with interesting CS profiles were whole genome sequenced. We find evidence that evolution in some drug conditions (fluconazole) produces adaptive mutants with predictable evolutionary tradeoffs. However, mutants that are adaptive in other drug conditions (radicicol) prove to have more diverse and less predictable tradeoffs. These experiments began to provide a quantitative understanding of the likelihood of evolutionary tradeoffs, including CS, how this likelihood changes depending on drug concentration, and whether patterns of CS are predictable across different mutants.

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Selection on plastic adherence leads to hyper-multicellular strains and incidental virulence in the budding yeast

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Many disease-causing microbes are not obligate pathogens; rather, they are environmental microbes taking advantage of an ecological opportunity. The existence of microbes that are not normally pathogenic, yet are well-suited to host exploitation, is an evolutionary paradox. One hypothesis posits that selection in the environment may favor traits that incidentally lead to pathogenicity and virulence, or serve as pre-adaptations for survival in a host (1). An example of such a trait is surface adherence. To experimentally test the idea of “accidental virulence”, replicate populations of two genetic backgrounds of the yeast, *Saccharomyces cerevisiae,* which can be an opportunistic pathogen, were evolved to attach to a plastic bead for hundreds of generations. As expected, plastic adherence ability increased over the course of the experiment. In all replicate populations, two other multicellular phenotypes also increased: biofilm formation and flor (floating mat) formation. A third multicellular phenotype, pseudohyphal growth, responded to the nutrient limitation, with the control populations also evolving. Thus, experimental selection led to the evolution of hyper-multicelluar strains that excelled at all of the assayed phenotypes. We tested whether virulence also increased using the greater wax moth model, *Galleria mellonella.* Larvae injected with evolved non-multicellular strains were significantly more likely to survive than those injected with evolved hyper-multicellular strains. Thus, selection on plastic adherence incidentally led to the evolution of enhanced multicellularity and increased virulence. Our results support the idea that selection in the environment for a trait unrelated to virulence can inadvertently generate opportunistic, “accidental” pathogens.

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Evolution of protein thermal stability between two thermally diverged *Saccharomyces* species

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Thermal adaptations are widespread in nature, but their molecular basis and evolution is not well understood. Thermal limits change little over the short timescales of laboratory experimental evolution, whereas substantial shifts have occurred naturally in some organisms, like thermophiles. Proteins from thermophilic bacteria are uniformly more resistant to thermal denaturation, but the molecular basis for this thermal stability and its contribution to thermotolerance has been hard to unravel. While differences in thermotolerance between closely related mesophiles are common, these smaller shifts may not require extensive stabilization of the proteome.

The *Saccharomyces* species diverged around 15 million years ago and have evolved substantial differences in their thermal growth profiles. Of the two most distantly related species in the group, *S. cerevisiae* grows at an upper thermal limit 8°C higher than that of *S. uvarum.* We used thermal proteome profiling of each species and their hybrid to determine whether they have diverged in their protein thermostabilities.

We find that a large fraction of *S. cerevisiae* proteins are more thermostable compared to their *S. uvarum* orthologs. While this pattern also holds true in the interspecies hybrid, the magnitude of thermostability differences between orthologs is reduced. We attribute the smaller differences in protein stability to the hybrid cellular environment having a stabilizing effect on the *S. uvarum* proteome, potentially mediated by protein – protein interactions. Our results show a proteome wide shift in protein thermal stability concomitant with divergence in thermal growth limits and suggest that thermal adaptation involves numerous changes of small effect.

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Quantitative genetics and biotechnological exploitation of novel yeast hybrids

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Interspecies hybridization increases the genetic variations in natural yeast populations, and it is also an important mechanism for the origin of novel lineages and adaptation to new environments. In the Saccharomyces genus, hybrids are readily found in nature and in many industrial applications including various fermentations to produce alcoholic beverages. However, interspecies hybrids are sterile and therefore an evolutionary dead end unless fertility is restored. In this study, we overcame infertility by creating tetraploid intermediates of *Saccharomyces* interspecies hybrids to allow continuous multigenerational breeding. We incorporated nuclear and mitochondrial genetic diversity within each parental species, allowing for quantitative genetic analysis of traits exhibited by the hybrids and for nuclear–mitochondrial interactions to be assessed. Using pooled F12 generation segregants of different hybrids with extreme phenotype distributions, we identified quantitative trait loci (QTLs) for tolerance to high and low temperatures, high sugar concentration, high ethanol concentration, and acetic acid levels. We identified QTLs that are species specific, that are shared between species, as well as hybrid specific, in which the variants do not exhibit phenotypic differences in the original parental species. Moreover, we could distinguish between mitochondria-type–dependent and –independent traits. This study tackles the complexity of the genetic interactions and traits in hybrid species, bringing hybrids into the realm of full genetic analysis of diploid species, and paves the road for the biotechnological exploitation of yeast biodiversity.

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Distinct patterns of aneuploidization impact the structure and stability of the *Saccharomyces cereivisae* genome

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The frequencies and patterns by which cells gain new genomic mutations profoundly shape their evolutionary trajectories and phenotypic potential. Recently, we established that several classes of large-scale structural genomic mutation, including whole chromosome copy number alterations (*e.g.,* aneuploidies), are acquired by cells via at least two distinct modes: the well-established neo-Darwinian pattern of gradual accumulation, during which single aneuploidies are acquired independently over time, and a burst-like pattern characterized by transient episodes of punctuated systemic genomic instability (PSGI), during which multiple aneuploidies are acquired simultaneously. The coexistence of such disparate tempos of aneuploidization has critical implications for our current paradigms of genome stability and evolution, yet, the incidence of each mode, as well as the molecular mechanisms underlying these different patterns of aneuploidization remain poorly understood. Using *Saccharomyces cerevisiae* cells, we have defined the frequencies at which gradual and pSGI-class aneuploidization events occur in populations, and in doing so have also characterized a third distinct class of aneuploidization. This third pattern is characterized by chromosome-specific uniparental disomy (UPD), a copy-neutral karyotypic alteration which results in the concurrent loss of one homolog and gain of the other. Interestingly, we have found that different yeast chromosomes display unique aneuploidization spectra, suggesting that chromosome-intrinsic features influence the propensity of a given chromosome to become aneuploid by a gradual, pSGI, or UPD-type mechanism. Together, these studies define a comprehensive model of aneuploidization, and inform on the intrinsic and extrinsic sources which may contribute to the stochastic patterns by which cells acquire *de novo* aneuploidies.

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A genetic screen uncovers sister chromatid cohesion without Pds5

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During DNA replication, the newly created sister chromatids are held together until their separation at anaphase. The cohesin complex is in charge of creating, and maintaining, sister-chromatid cohesion (SCC) in all eukaryotes. In yeast cells, cohesin is composed of two elongated proteins, Smc1 and Smc3, bridged by the kleisin Mcd1/Scc1. The latter also acts as a scaffold for three additional auxiliary proteins, Scc3/Irr1, Wpl1/Rad61 and Pds5. Although the HEAT-repeat protein Pds5 is essential for cohesion, its precise function is still debated.

Deletion of the *ELG1* gene, encoding a PCNA unloader, can partially suppress the temperature-sensitive *pds5-1* allele, but not a complete deletion of *PDS5.* We carried a genetic screen for high copy number suppressors, and another for spontaneously arising mutants, able to allow survival of a *Δpds5 Δelg1* strain. Our results show that cells remain viable in the absence of Pds5 provided that there is both an increase in the level of PCNA on chromatin, and an elevation in the level of Mcd1. Thus, our results delineate a double role for Pds5 in protecting the cohesin ring, and interacting with the DNA replication machinery.

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Uncovering natural histories of mutator alleles in budding yeast

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Mutations provide essential raw material for evolutionary change. Genetic factors that increase mutation rates, i.e. mutator alleles, have been observed to arise during experimental evolution, facilitating adaptation to the lab environment. However, little is known about how prevalent mutator alleles are in natural populations and how they have historically contributed to evolution due to the rarity of mutations under normal conditions. To tackle this challenging question, we have established a framework that utilizes the mutation spectrum—the relative frequencies of different types of mutations, calculated from natural polymorphisms—to determine the potential historical impacts of *Saccharomyces cerevisiae* mutation rate modifiers that affect certain mutation types disproportionally. We combined this with efficiently accumulating *de novo* mutations in a reporter gene using a modified fluctuation assay of natural isolates to identify *S. cerevisiae* populations that have experienced recent mutation rate and spectrum changes. We discovered a 10-fold range of mutation rate variation among 16 haploid strains from diverse populations. Two strains from the Mosaic beer clade have excess C>A mutations in both *de novo* and rare natural polymorphisms, indicating a recent occurrence of at least one mutator allele common to the two strains. We further identified a mutator allele in *OGG1* that partially explains these strains' mutator phenotype. Our mutation spectrum analysis of polymorphisms also indicates that additional mutators have likely been influencing the evolution of natural populations beyond the Mosaic beer strains. We discovered that strains from the African beer population are even more conspicuous mutation spectrum outliers, and a subset of French dairy strains show intermediate mutation spectra between the African beer and the rest of the strains. Analysis of these French dairy strains suggests that there are likely mutator alleles introgressed from African beer strains affecting the subsequent mutation spectra. We have engineered a reporter into the African beer and French dairy strains for the modified fluctuation assay, and we are measuring *de novo* mutation spectra in these strains to test this hypothesis. In summary, our framework has proven useful in identifying mutator alleles in natural populations of budding yeast, and will reveal how mutator alleles contribute to evolution.

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High-throughput single cell sequencing with linear amplification to study yeast recombination

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Conventional methods for single cell genome sequencing are limited with respect to uniformity and throughput. Here we describe "sci-L3", a high-throughput, high-coverage single cell sequencing method that combines single cell combinatorial indexing ("sci") and linear ("L") amplification. The sci-L3 method adopts a unidirectional 3-level ("3") indexing scheme that minimizes amplification biases while enabling exponential gains in throughput. At 20% genome coverage, we applied sci-L3 to profile the genomes of 10,000 single cells from W303/YJM789 yeast hybrid diploids, with homozygous mutations in the *SGS1* gene, mapping tens of thousands of loss-of-heterozygosity (LOH) events resulting from spontaneous mitotic recombination. In highly-covered cells, we were able to map interstitial gene conversion events of 10 kb in single cells. We are developing computational methods 1) to call LOH with hidden Markov model with K-segment constraint; 2) to call copy number basing on digital DNA counting with transposomes-mediated genome fragmentation; and 3) to dissect clonal structures and to build lineage relationship between and within colonies. We anticipate that the sci-L3 assays and computational suites can be applied to fully characterize recombination landscapes, to reveal heterogeneity regarding genome instability, and to other goals requiring high-throughput, high-coverage single cell genome sequencing.

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DNA replication protein Cdc6 interacts with mitotic cyclin Clb2 to arrest cell cycle at metaphase under re-replication stress

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A stepwise assembly of the pre-replicative complex is required to initiate DNA replication. The pre-RC includes Orc1-6, Cdc6, Cdt1 and Mcm2-7, which is bound on the origin of DNA replication during G1 phase. Cdc6 protein level accumulates in mitosis when it is tightly bound by mitotic cyclin Clb2. The Cdc6-Clb2 interaction is mediated though Cdc6 N-terminal phosphorylation. This protein interaction ensures that: 1. Cdc6-Clb2 interaction shields Cdc6 phospho-degron, thereby stabilizing Cdc6 in mitosis. 2. Cdc6-Clb2 binding prevents premature origin licensing. 3. Cdc6 also inhibits Clb2 function; e.g. Clb2-Cdk1 activity is inhibited when Cdc6 is bound.

Recently, we reported that PP2A-Cdc55 phosphatase dephosphorylates Cdc6-T7 sites to release Clb2 in late mitosis. The PP2A dependent Cdc6 dephosphorylation triggers mitotic exit and origin licensing. In this study, we show that Cdc6 is tightly bound to Clb2 when DNA re-replication is induced, leading to metaphase arrest with short spindles. The metaphase arrest was partially reversed in *CDC6-lxf*, a binding mutant to Clb2. When Cdc6 T7 site is more phosphorylated in *cdc55* deletion mutant, S-phase progression was significantly impaired. It indicates that Cdc6-T7 phosphorylation status and Cdc6-Clb2 interaction inhibits S-phase, origin licensing and mitotic Cdk1 activity under re-replication stress. Furthermore, spindle assembly checkpoint and Swe1 were dispensable for metaphase arrest in the re-replication mutants.

We hypothesize that DNA re-replication enhances Cdc6-Clb2 interaction to arrest the cell cycle in mitosis, which may serve as a novel cell cycle checkpoint. Such a mechanism ensures faithful genomic integrity during DNA re-replication and DNA damage.

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A Humanized Yeast Platform Defines *in vivo* Targets of APOBEC3C Mutagenesis

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The APOBEC3 family of cytidine deaminases is involved in the restriction of viruses and endogenous retroelements in primates by DNA and mRNA editing. More recently, APOBEC3 family members, particularly APOBEC3A and APOBEC3B, were identified as prominent mutators of human cancer genomes. Recent data from my research group suggests that APOBEC3C is an additional mutator of nuclear genomes, and so could be a cancer genome mutator. I expressed human APOBEC3C in yeast in order to define its chromosomal DNA substrate. Results from whole genome sequencing of APOBEC3C strains after accumulation of mutations define a mutation signature that is distinct from that of APOBEC3A or APOBEC3B. Additionally, APOBEC3C preferentially deaminates deoxycytidines that are proximal to DNA replication origins without showing the lagging strand bias that is apparent for APOBEC3A and APOBEC3B. Genome-scale genetic interaction screens indicate that ssDNA is the likely in vivo substrate for APOBEC3C, and that APOBEC3C mutants lacking deaminase activity are not genetically inert. The unique properties of APOBEC3C revealed using the humanized yeast platform will aid in analysis of cancer genomes.

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CRI-SPA – a mating based, CRISPR-Cas9 assisted method for high-throughput genetic modification of yeast strain libraries.

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Combining genetic features can give rise to unpredictable genetic interactions. These interactions challenge our understanding of gene function and complicate genetic engineering efforts. For example, an engineered phenotype in a microbial strain might be lost upon addition of a single genetic modification. Conversely, two modifications can happen to combine synergistically producing an unforeseen enhanced phenotype. A simple means to systematically map genetic interactions in a genetic engineering context could help identify synergistic combinations and guide designs towards phenotypic maximas. To this end, we have developed CRI-SPA, a method enabling the high-throughput screening of interactions between a genetic feature and the background of its host in *Saccharomyces cerevisiae*. CRI-SPA employs arrayed mating, CRISPR-Cas9-induced gene conversion and Selective Ploidy Ablation to transfer the feature from a donor strain into a library of recipient strains within a week. We demonstrate the power of CRI-SPA by introducing four genes responsible for the production of the plant product betaxanthin in one step into yeast knock-out collection >4800 strains). The screen identifies new gene candidates and the data provides a genome-wide overview of the genetic requirements for betaxanthin production in yeast. Dealing with genetic interactions is a critical challenge for microbial engineering. CRI-SPA proposes to harness them to our own advantage.

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Yeast-based screening system to identify and optimize the anti-sense oligos that promote targeted base-editing in mRNA molecules by the ADAR enzymes

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Adenosine deaminase acting on RNA (ADAR) proteins are endogenous enzymes catalyzing the deamination of adenosine nucleotides to inosines, which are then read as guanosines during translation. This ability to re-code makes ADAR an attractive therapeutic tool to correct genetic mutations and reprogram genetic information at the mRNA level. ADARs carry out their activity in regions of dsRNA formation, in this respect, an approach utilizing the endogenous ADARs, and guiding them to a selected target has a huge potential. Indeed, different studies have reported several site-directed RNA editing approaches for making targeted base changes in RNA molecules. The basic strategy has been to use an antisense oligo (ASOs) that hybridizes and forms a dsRNA structure with the desired RNA target. While these structures directed the ADAR enzyme and induced on-target editing events, off-target editing was extensive, both within the targeted message and across the entire transcriptome of the transfected cells. These results imply that more effective guides would be useful for better redirecting endogenous ADAR, however, the determinants that govern such activity are largely unknown. Since genomes that express one or more ADAR proteins have been subjected to long evolutionary selective pressures, which affected which sites can be edited and which cannot, it has been hard to establish exact rules determining how the editing enzymes choose their sites. Introducing them into a heterologous system allows us to establish the rules from zero. This was achieved by the exogenous expression of the ADAR proteins in the yeast *Saccharomyces cerevisiae*, an organism whose origins precede the emergence of ADARs but can express the human enzyme. The cells were transformed with a library of plasmids expressing millions of ASOs and engineered to uncover ASOs that are particularly more efficient in the editing of selected targets,

To demonstrate the feasibility of this screening system we identified and optimized ASOs sequences that enabled the recruitment of endogenous ADAR for the efficient repair of nonsense mutations in the genes *TRPM1,* and *USH2A* that cause common inherited retinal diseases, a major cause of blindness. The identified ASOs were tested and validated in human cell lines, and currently form the basis for better future designs including applying this genetic therapy to the appropriate knock-in mouse models, retinal organoids, and ultimately in human patients.

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Powerful Genome-wide Screening in any Species or Strain through Profiling of Hermes Insertion Mutations

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Research on non-model yeasts such as the human pathogen *Candida glabrata* has been hampered by the lack of genetic tools, such as barcoded gene knockout collections. Such collections would be useful for genetic screens of virulence, pathogenicity, and drug resistance mechanisms that are not possible in *Saccharomyces cerevisiae*. We present a fast, easy, inexpensive, and comprehensive method for generating and quantitatively analyzing complete gene knockout collections in virtually any haploid yeast species. Hermes Insertion Profiling (HIP) involves random insertional mutagenesis using the Hermes transposon followed by Illumina DNA sequencing of insertion sites *en masse*. In *S. cerevisiae*, *C. glabrata*, and its non-pathogenic relative *N.delphensis*, one insertion every ~20 bp was readily achieved, indicating saturation mutagenesis. Essential genes, ideal targets for novel antifungals, were easily identified in all three species based on the paucity of recovered insertion sites and read depth relative to adjacent non-essential genes. The essentialomes of all three species showed strong overlap as well as distinct differences that reflect the evolutionary trajectories of the different yeasts. Using the large pools of insertion mutants, we performed genome-wide genetic screens for genes that regulate susceptibility to fluconazole, a front-line fungistat used to treat yeast infections. The screens yielded Pdr1 transcription factor and its target Pdr5/Cdr1, a fluconazole-efflux pump, as major determinants of fluconazole resistance. By generating new pools of Hermes insertion mutants in *pdr1*Δ and *cdr1*Δ mutants of *C. glabrata*, we define numerous upstream regulators of Pdr1 and Cdr1 that strongly alter susceptibility to fluconazole. Surprisingly, the findings suggested that deficiencies in 60S ribosome biosynthesis or function activates Pdr1 and Pdr5/Cdr1. The translation inhibitor cycloheximide strongly activated Pdr1 dependent on ribosomal binding. This data argues against the dogma of Pdr1 functioning as a direct receptor for xenobiotics and suggests that Pdr1 indirectly senses translation stress and several other stresses. The connection between translation stress and Pdr1 signaling was previously overlooked as most ribosomal subunits are essential and complete deletion mutants were not possible, while HIP is able to create hypomorphs which are not easily generated by other methods.

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A novel Cas3 base-editor for efficient directed evolution of complex metabolic pathways in *S. cerevisiae*

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Directed evolution is a common strategy to obtain superior industrial microbes. Because the relatively low supply of beneficial mutations limits the efficiency of directed evolution, various strategies are used to increase the occurrence of mutations. These include mutagens such as UV and EMS, which increase the mutation rate throughout the genome, as well as more directed approaches like error-prone PCR and repeated transformation with variable oligonucleotides. However, each of these strategies comes with important limitations. Increasing the global mutation rate implies the risk of detrimental hitchhiker mutations that reduce the strain's performance, while more directed approaches often only target one or few very specific loci, for example encoding an active site.

Here, we report the development of a Cas3-based strategy to increase the rate of random mutations within specifiable, large genomic regions, without affecting the rest of the genome. The tool combines CRISPR-guided targeting and inducible base-editors that feature a cytidine deaminase fused to Cas3, the signature enzyme of class 1 type I-E CRISPR-Cas systems. The helicase activity of Cas3 unwinds large stretches of double-stranded DNA (10-100 kb) into single-stranded DNA, providing substrate for the cytidine deaminase. The large action radius of Cas3 allows the deaminase to mutate extended regions of DNA, a feat that is not possible with other base-editing tools. Our results show that the Cas3 base-editor can significantly increase cytidine deaminations within at least 12 kb downstream of the target site. The base-editor introduced an average of 0.35 cytidine deaminations per kb with a mutation occurring every 1-1.5 kb, on average. Whole genome sequencing reveals a greater than 150-fold increase in cytidine deaminations within the targeted window compared to rest of the genome. The extended reach of our Cas3 base-editor as well as its targetability enables the directed evolution of entire heterologous pathways in *S. cerevisiae* as we have demonstrated by using it to increase the efficiency of lycopene production in yeast. Hence, the new tool bridges the current technological gap between genome-wide and narrowly localized mutagenesis and proves an ideal tool for the optimization of complex biosynthetic pathways through directed evolution.

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Transformation-mediated chromosome synthesis and replacement in eukaryotic cells

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Chromosome synthesis is a powerful approach for studying how genomes give rise to cellular life and its diversity. However, de novo chromosome synthesis is costly and laborious, limiting its application to many biological problems. Here, we report a method for cloning segments of natural chromosomes and assembling them into synthetic chromosomes that replace the endogenous chromosomes in yeast cells. Using a CRISPR/Cas9-mediated transformation-associated recombination ('TAR') cloning strategy, we both capture natural molecules and append 'adapters' to them that specify how these molecules will recombine in living cells. Using this technique, we generate 27 chimeric chromosomes with genetic material from multiple strains and species, 9 programmably restructured chromosomes, and a chromosome with

many genes intentionally deleted. This clone-and-assemble methodology is a versatile approach that significantly expands the types of genetics and genomics research that can be addressed through chromosome synthesis.

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Gene Presence Networks Predict Novel Respiratory Genes from Multiple Losses of Mitochondrial Complex I across Yeasts

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The yeast *Saccharomyces cerevisiae* is a powerful model for studying mitochondrial genetics but the evolutionary history of this species constrains its use in studying the canonical NADH-ubiquinone oxidoreductase, Complex I. In *S. cerevisiae* and related species this complex has been replaced by an alternative system resulting in loss of both nuclear- and mitochondrial-encoded components. The rapid expansion of high quality genomes of related budding yeasts provides new opportunities to study Complex I evolution and function. By assembling over 150 novel yeast mitochondrial genomes (mtDNAs) and analyzing over 350 mtDNAs, we found that the mitochondrial-encoded components of Complex I have likely been lost independently five times in budding yeast evolution. To determine which genes were consistently lost across all losses of Complex I, we examined patterns of gene presence and absence across the nuclear genomes of these species and developed a network of associations based on mutual information. Searching the network for genes lost concomitantly with Complex I revealed 44 candidate respiratory genes. These genes are heavily enriched for known Complex I subunits, as well as several previously uncharacterized genes. Gene deletion mutants from high-throughput studies in *Candida albicans* suggest these genes are phenotypically important but, surprisingly, are generally not essential despite the essentiality of mtDNA in these yeasts. We hypothesize that many yeast species are robust to moderate perturbations in respiration and Complex I in particular due to partial redundancy in electron transfer with Complex II which may have facilitated the loss events. Further knockouts in *C. albicans* and *Komagataella pastoris* are currently underway to validate the role of these genes in Complex I function. Network analysis of gene presence associations across yeasts provides a promising method to assess the function of a variety of uncharacterized genes in this group.

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Impact of structural variants on gene expression variations in a yeast natural population

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Structural variants (SVs) are an important source of genetic variation in a large number of organisms such as the *Saccharomyces cerevisiae* yeast species. Nevertheless, they are still poorly explored at the population level and their functional effects are not well understood. In fact, in the last decades the genotype-phenotype relationship was dissected using diverse strategies such as genome-wide association studies (GWAS) without taking SVs into account. This is most likely one of the reasons why the genetic variants associated with complex traits usually explained a small fraction of the phenotypic variance.

To have a deep insight into the functional impact of SVs on a specific molecular trait, namely gene expression variation, we first decided to obtain a population to perform GWAS. We generated a half diallel panel between 28 yeast natural isolates leading to a population of 406 hybrids. Using the Oxford Nanopore long read sequencing strategy, we then established the exhaustive catalog of the SVs present in the parental isolates, allowing us to infer the SV contents in the generated hybrids. In parallel, we quantified gene expression and determined the transcriptome of the 406 hybrids using RNA sequencing. Altogether, these two datasets allowed us to exhaustively determine the SVs having an effect on the gene expression level (SV-eQTL) in cis and trans via genome-wide associations. In addition, we could also precisely estimate the impact of single nucleotide polymorphism on gene expression variation (SNP-eQTL) in the same population. Our results clearly showed that

SV-eQTL increased the fraction of the variance explained for a large number of traits, highlighting their functional effects. Overall, our study was able to characterize the details underlying many aspects of the impact of SVs on gene expression and how they affect gene regulation, and therefore complex traits.

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Pararesistance: a mutation-independent mechanism of antifungal drug resistance

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Drug resistance is a major cause of treatment failure in infectious diseases and a global public health crisis. Although much research has been focused on identifying mutation-based mechanisms underlying drug resistance, cell-to-cell variation in drug response may be a hidden force that promotes the rapid adaptation to drug-induced stress. Recent studies suggest that an effective strategy for adapting to fluctuating environments is via a heritable but reversible phenotypic state. However, the mechanisms that govern the establishment and maintenance of such states remain poorly characterized. Here, we describe a mutation-independent mechanism of stress adaptation that accelerates the acquisition of drug resistance in a leading human fungal pathogen, *Candida albicans*. We discovered that transiently exposing a clonal

population to the widely prescribed antifungal fluconazole elicits a sustained protective response in a subpopulation of cells. To distinguish from genetic-based resistance and epigenetically-encoded persistence and tolerance, we coined the term 'pararesistance' to describe a high-frequency adaptive state that is heritable and reversible. We further discovered that perturbing the proteostasis network strongly impacts the establishment of pararesistance: its acquisition can be triggered by the transient exposure to unfolded protein stress (e.g. Hsp90 inhibitor radicicol and ADP-ribosylation factor inhibitor brefeldin A), and blocked by small molecules that disrupt protein phase separation or aggregation (e.g. guanidine hydrochloride and 1,6-hexanediol). Additionally, we demonstrated that regardless of inducing cue, many of the pararesistant isolates constitutively upregulate a number of multidrug transporters such as *CDR1*. Consistent with this finding, phenotypic characterization of 62 pararesistant isolates across 20 different growth conditions revealed that pararesistance confers resistance to a number of Cdr1 substrates in addition to fluconazole, including brefeldin A and terbinafine. These results indicate that pararesistance confers resistance to fluconazole at least in part via upregulation of drug efflux. Together, this work presents a new paradigm for understanding non-genetic mechanisms that drive the rapid evolution of drug resistance, establishing a conceptual framework for developing novel therapeutic strategies that target evolutionary processes.

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The evolution of killer toxin resistance in the opportunistic human pathogen *Candida glabrata*

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Killer toxins are antifungal proteins produced by many species of fungi. The opportunistic fungal pathogen *Candida glabrata* is uniquely susceptible to killer toxins produced by *Saccharomyces* yeasts compared to other *Candida* and *Nakaseomyces* yeast species. Specifically, the ionophoric K1 killer toxin successfully inhibited the growth of 135 diverse clinical and environmental strains of *C. glabrata*. Due to the absence of killer toxin resistance in *C. glabrata*, it was possible to investigate whether *C. glabrata* could evolve K1 resistance. Of the K1 toxin-resistant clones isolated, we found that growth rate and morphology were largely unaffected. However, resistant clones had increased sensitivity to cell wall and membrane damaging agents and reduced virulence in a *Galleria mellonella* larvae model of yeast pathogenicity. Whole-genome sequencing revealed novel genes and polymorphisms involved in K1 resistance, including those critical for vesicle trafficking (GARP complex) and cell wall morphogenesis (RAM network). Moreover, we have observed changes in membrane and organelle composition associated with K1 resistance and alterations in the localization and abundance of the GPI-anchored K1 membrane receptor (Kre1p). This work provides a unique mechanistic insight into K1 toxin resistance in *C. glabrata* and how an *S. cerevisiae* killer toxin can be broadly antifungal to an opportunistic human pathogen.

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Extensive sampling of *Saccharomyces cerevisiae* in Taiwan reveals ecology and evolution of predomesticated lineages

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The ecology and genetic diversity of model yeast *Saccharomyces cerevisiae* prior to human domestication remain poorly understood. Taiwan is regarded as part of this yeast's geographic birthplace where the most divergent natural lineage was discovered. Here, we extensively sampled the broad-leaf forests across this continental island to probe the ancestral species diversity. We found that *S. cerevisiae* is distributed ubiquitously at low abundance in the forests. Whole-genome sequencing of 121 isolates revealed nine distinct lineages that diverged from Asian lineages during the Pleistocene, when a transient continental shelf land bridge connected Taiwan to other major landmasses. Three lineages are endemic to Taiwan and six are widespread in Asia, making this region a focal biodiversity hotspot. Both ancient and recent admixture events were detected between natural lineages and a genetic ancestry component associated with isolates from fruits was detected in most admixed isolates. Collectively, Taiwanese isolates harbor genetic diversity comparable to that of the whole Asia continent, and different lineages have coexisted at a fine spatial scale even on the same tree. Patterns of variations within each lineage revealed that *S. cerevisiae* is highly clonal and predominantly reproduces asexually in nature. We identified different selection patterns shaping the coding sequences of natural lineages and found fewer gene family expansion and contractions which contrast with domesticated lineages. This study establishes that *S. cerevisiae* has rich natural diversity sheltered from human influences, making it a powerful model system in microbial ecology.

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Domestication of *S. cerevisiae* in baking: a peek into the evolutionary history of your sourdough starter

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Humans have relied on yeasts to ferment dough for breadmaking for thousands of years. The process of breadmaking presents several unique selection pressures for yeast, including osmotic stress, metabolism and fermentation of complex sugars, selection for aromas and tastes, and competition with a mixed microbial community, all of which have influenced the domestication history of the most prevalent baking yeast, *Saccharomyces cerevisiae*. Our objective is to understand how these selection pressures in the breadmaking environment have shaped the genomes of baking strains, with a broader goal of better understanding *S. cerevisiae* prevalence in fermentation environments globally. We leverage a library of sourdough starters collected from home bakers across North America. We isolated and sequenced the genomes of approximately 50 *S. cerevisiae* strains, and find that North American baking strains are not monophyletic, spanning divergent clades across the *S. cerevisiae* phylogeny. We have phenotyped these strains for several baking traits, documenting significant differences in growth rate in maltose, the most prevalent sugar in dough, and in the rate of dough rise, with numerous strains

outperforming commercial baking yeast. Ploidy ranges from diploid to tetraploid with prevalent aneuploidy, and we highlight several important gene amplifications and losses. Our results suggest that selection on key baking traits has resulted in genotypic and phenotypic convergence in sourdough yeasts.

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The E2 ubiquitin conjugase Rad6 is a master regulator of translation during oxidative stress

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Cellular adaptation to oxidative stress relies on a refined regulation of gene expression at the transcriptional and translational levels. Our group discovered that ribosomes are heavily ubiquitinated in response to oxidative stress and that this modification affects ribosome structural conformation and is critical for the regulation of the elongation stage of translation. However, we had limited understanding on the mechanisms triggering ribosome recognition and ubiquitination following oxidative stress induction. Here, we showed that the E2 conjugase enzyme Rad6 constitutively binds to ribosomes and polysomes. Cryo-EM and molecular modeling revealed that acidic and hydrophobic residues are key for Rad6 interaction with the 40S subunit of the ribosome. Moreover, we showed that Rad6 is redox regulated in response to stress. Proteomics analysis revealed that Rad6 forms a reversible disulfide with the E1 Uba1, which in a negative feedback mechanism, limits the amount of ribosomal ubiquitination. In addition, we showed that Rad6 is required for the reprogramming of translation in response to stress. Mass spectrometry and immunoblotting analyses showed that deletion of *RAD6* leads to continuous and aberrant protein production, which is detrimental for cellular growth. Finally, RiboSeq analysis of wild-type cells demonstrated a prominent representation of ribosome pausing at specific sequence motifs under stress that are completely abolished in the absence of *RAD6.* Collectively, our data support a new mode of translation regulation mediated by Rad6 and ubiquitination of ribosomes, which determines cellular antioxidant capacity and supports its resistance to oxidative stress.

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Pathogenic missense mutations in genes encoding structural subunits of the RNA exosome complex cause distinct transcriptomic changes as well as altered interactions with RNA exosome cofactors

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RNA exosomopathies, a growing family of tissue-specific diseases, are linked to missense mutations in genes encoding the structural subunits of the highly conserved exoribonuclease complex, the RNA exosome. This RNA exosome processes and/or degrades nearly all classes of coding and non-coding RNAs with a critical role in producing mature rRNA. The 9-subunit core RNA exosome, which was first assessed in budding yeast, forms a barrel-like structure composed of three S1/KH cap subunits (yeast Rrp4/40/Csl4; human EXOSC2/3/1) and a lower ring of six PH-like subunits (yeast Rrp41/42/43/45/46/Mtr3; human EXOSC4/7/8/9/5/6) that associates with a 3'-5' exoribonuclease (yeast Rrp44; human DIS3/DIS3L). Missense mutations in the cap subunit gene *EXOSC2* cause the novel syndrome SHRF (Short stature, Hearing loss, Retinitis pigmentosa and distinctive Facies). In contrast, missense mutations in the cap and core subunit genes, *EXOSC1, EXOSC3, EXOSC5, EXOSC8* and *EXOSC9,* cause neurological defects (pontocerebellar hypoplasia type 1 [PCH1] and Cerebellar Ataxia, Brain Abnormalities, and Cardiac Conduction Defects [CABAC]). We hypothesize that differences in disease phenotypes could reflect distinct defects in RNA exosome function, including misprocessing/accumulation of specific RNA exosome target RNAs and/or disrupted interactions with RNA exosome cofactors. To gain insight into the functional consequences of the *EXOSC2/3/5* mutations, causing SHRF, PCH1 and CABAC, respectively, we utilized *Saccharomyces cerevisiae* to generate the corresponding mutations in the orthologous *Saccharomyces cerevisiae* genes *RRP4/40/46*. We find that *rrp* variants have differential cell growth and distinct molecular defects in complex integrity of the RNA exosome, which could shed light on the diversity of exosomopathy disease phenotypes. To further explore defects in RNA exosome function, we performed RNA-Seq analysis and detect differential changes in specific pools of target RNAs when comparing the three exosomopathy models. Given the critical role of the RNA exosome in 3'-5' processing of rRNA, we also hypothesized that RNA exosomopathy mutant models might show defects in translation. We employed a reporter system to assay different aspects of translational quality control such a frameshifting, stop codon readthrough and miscoding. Surprisingly, we found that different *rrp* variants show distinct defects in translational fidelity. Furthermore, we detect specific negative genetic interactions between RNA exosome cofactor mutants and a *rrp4* variant, suggesting destabilization between the essential RNA helicase Mtr4 and the RNA exosome when harboring these SHRF exosomopathy mutations. These studies provide the first comparative analysis of RNA exosomopathy mutations and provide a basis of how to assess this growing and diverse family of disease mutations *in vivo*.

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Dynamic Phosphorylation of Eukaryotic Translation Initiation Factor 4A Couples Cell Growth and Translation

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The eukaryotic translation initiation factor 4A (eIF4A) supports mRNA recruitment to the ribosomal preinitiation complex, thus controlling cell-wide protein synthesis. Several conserved Serine and Threonine residues of eIF4A reside near the catalytically-important DEAD motif and were found to be phosphorylated in large-scale studies. To further dissect translational control by eIF4A, we analyzed changes in phosphorylation that take place during cell cycle arrest and in response to glucose deprivation, states known to show dramatic differences in translation activity, and analyzed the effects of several events on eIF4A function. Surprisingly, we detected only phosphorylated peptides for a site immediately adjacent to the DEAD-box motif during G1/S arrest, and found no phosphorylation of that residue during G2/M arrest, suggesting the diametrically opposed phosphorylation status may affect eIF4A functions needed for proper transitions through the cell cycle. Mutation of this site to phosphodeficient Ala led to decreased growth and translation rates, suggesting phosphorylation of eIF4A is needed for more robust growth and translation during G1/S phase. We found additional residues in eIF4A displayed increased phosphorylation during mitosis and glucose deprivation, and mutation of these sites to phosphomimetic (Asp/Glu) led to ~90% of cells arresting during mitosis as large mother-daughter pairs incapable of division. The same mutations abolished eIF4A RNA-binding in vitro and decreased affinity for eIF4G in yeast. Together these results suggest that dynamic phosphorylation of alternative sites in eIF4A serves both to stimulate translation during periods of rapid growth, or to rapidly transition eIF4A to an inactive state when a reduction in translation is necessary. Dephosphorylation of inhibitory residues of eIF4A is required for cells to complete division, suggesting an important functional role for eIF4A in coupling translation to cellular growth states.

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Investigating the role of G4 DNA in transcription control in budding yeast

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G-quadruplex or G4 DNA is a non-B secondary DNA structure that consists of multiple guanine tetrads stacking on top of each other. G4 DNA can alter cellular functions such as replication, transcription, and induce genomic instability. A potential G4-forming DNA sequence located in a gene promoter can affect the transcription outcome. To further understand how G4 DNA regulates gene transcription, we identified 37 genes in the genome of *Saccharomyces cerevisiae* that have G4 motif in their promoters by using QGRS mapper. Interestingly, among these, there are 20 genes that have G4 motifs overlapping with the Stress Response Element (AGGGG - STRE), which is the binding site of stress response transcription factor Msn2 or Msn4. When G4 structures are stabilized with G4 ligands, 19 of these genes with both G4 motifs and STRE are up-regulated including 13 of previously identified Mns2/4 target genes, 5 genes are down-regulated including 3 of previously identified Mns2/4 target genes. Deletion of *MSN2* and *MSN4* genes reduced the transcriptional elevation caused by the G4 ligand treatment at these Msn2/4 target genes including *ATG20*, *ATG39*, *TPS1*, and *TSL1*. Surprisingly, we found that Msn2 not only binds to STRE site in double-stranded DNA but also binds to G4 structures formed by single-stranded DNA oligos with sequences from *ATG20*, *ATG39*, *TPS1*, or *TSL1* promoters *in vitro*. In addition, we also found that Msn2 binds to G4 structures that do not have overlapping STRE site. These data suggest that Msn2/4 binds to G4 DNA formed at gene promoters and modulate the transcription. Overall, we hypothesize that G4 DNA formation plays an important role in transcription regulation of some stress response genes through recruiting Msn2 or Msn4 transcription factor.

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Genetic interactions between cyclin C-Cdk8 and COMPASS regulate yeast meiosis

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Meiosis is a specialized cell division characterized by one round of DNA replication, genetic recombination and two rounds of division to yield four haploid gametes. In the budding yeast *Saccharomyces cerevisiae*, meiotic induction occurs when Mat a/a diploids are starved for both nitrogen and fermentable carbon, initiating a meiotic transcriptional program that is generally divided into three stages termed early, middle, and late. Recent work has revealed the presence of meiosis-specific noncoding RNAs termed MUTs that may be key regulators of gamete formation. Extensive and complex interactions between the RNA pol II holoenzyme and post-translational histone modifications are key regulators of coding and noncoding transcription. The RNA pol II holoenzyme is organized into two functionally distinct subcomplexes; core mediator and the Cdk8 kinase module (CKM). The CKM, composed of Med12, Med13, cyclin C, and Cdk8, represses early meiotic gene transcription and its inactivation is a key step for meiotic gene induction. Previous research in our laboratory demonstrated that cyclin C-Cdk8 can regulate transcription by antagonizing locus-specific recruitment of the histone H3K4 methyltransferase Set1. Precise H3K4 me catalysis relies upon specific interactions between Set1 and COMPASS. We recently reported meiosis-specific functions for the COMPASS catalytic core subunits Swd1 and Swd3, which are required for all H3K4 me levels. This study indicated that Swd1 and Set1 are required for cellular progression from early to middle meiosis, while Swd3 is more important for late meiotic processes. In the current investigation, we determine how genetic interactions between cyclin C-Cdk8 and the COMPASS complex impact meiosis. We found that cyclin C-Cdk8 is epistatic to COMPASS during meiosis as mutations in either cyclin C or Cdk8 cause cells to bypass key steps in early meiosis that require COMPASS complex activity. To identify specific transcriptional targets regulated by both cyclin C and Set1, we performed stranded RNA-seq analyses on yeast harboring single or double cyclin C and Set1 mutants in a meiotic time course experiment. These data revealed that

while protein coding transcripts show minor differences across all samples, MUTs are inhibited by cyclin C in a Set1-dependent manner. This suggests that genetic interactions between cyclin C-Cdk8 and Set1 are critical for both meiotic completion and the appropriate timing of MUT expression.

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Forging connections between threonine metabolism and chromatin regulation

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Traditionally, proteins have been thought to have only one primary function. However, recent research has brought attention to multi-functional proteins known as “moonlighters.” As more moonlighters are recognized and their new functions characterized, links are being created between important biological processes that were previously thought to be unrelated.

Two biological pathways are critically important for cellular homeostasis: amino acid metabolism and chromatin dynamics. Amino acids, the building blocks of proteins, can be utilized in protein synthesis and broken down to fuel other biological pathways. Chromatin, a complex made of proteins and DNA, is critical in ensuring that genes are turned on and off at appropriate times and places. When either of these processes is disrupted, developmental defects or diseases are unfortunately likely outcomes.

Increasing evidence demonstrates that the processes of amino acid metabolism and chromatin dynamics can be intertwined in functionally significant ways. Our work initially focused on homoserine dehydrogenase (Hom6) which is known to catalyze a key step in the synthesis of homoserine, a precursor to the amino acids methionine and threonine.

We discovered previously uncharacterized chromatin-based roles for Hom6 in both ribosomal DNA (rDNA) regulation and DNA repair. Interestingly, when supplemented with excess threonine, defects in rDNA silencing are suppressed in *hom6Δ* strains. This phenotypic rescue is specific to threonine as rDNA silencing defects remain unchanged when excess methionine is added to the growth media. In addition, threonine specifically regulates rDNA silencing in yeast, as *hom6Δ* silencing phenotypes at other loci were not affected by excess methionine or threonine. To our knowledge, this is the first report of a connection between the threonine biosynthetic pathway and the regulation of rDNA silencing. *hom6Δ* cells are also sensitive to treatment with a variety of DNA damaging agents indicating that Hom6 may promote DNA repair through multiple mechanisms.

Ultimately, the multi-faceted roles of threonine will be key in understanding the connections between important metabolic processes and chromatin biology. Similarly, expanded identification and characterization of moonlighters participating in both of these vital processes holds the promise of yielding diverse insight into gene regulation, genomic stability, and evolution.

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Pulse labeling reveals the tail end of protein folding by proteome profiling

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Accurate and efficient folding of nascent polypeptides into their native state requires support from the protein homeostasis network. We posit that certain proteins in cells might be particularly susceptible to misfolding and aggregation before reaching a state that confers solubility. Hence, we probed which newly translated proteins are thermo-sensitive and aggregate under heat stress using pulse-SILAC mass spectrometry. We found a unique group of proteins that is highly sensitive to stress when newly synthesized but not once matured. These proteins are abundant and highly structured. We reason that there may be a trade-off where some proteins require more time to mature and complete folding to then potentially reach a metastable conformation. Indeed, newly translated thermo-sensitive proteins have longer half-lives. In addition, they display a tendency to form β-sheet secondary structures, a configuration which typically requires more time for folding, and are enriched for chaperone binding motifs, suggesting a higher demand for chaperone-assisted folding. These polypeptides are also more often components of stable protein complexes in comparison to other proteins. Using limited proteolysis coupled to pulse-SILAC, we show that these thermo-sensitive proteins display more exposed domains or regions when newly translated in comparison to other proteins. Combining this evidence suggests that there exists a specific subset of proteins in the cell that is particularly vulnerable to misfolding and aggregation following synthesis and before reaching the native state.

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Genetic Variation in the Ubiquitin System Creates Complex, Pathway-Specific Effects on Proteasomal Protein Degradation

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Genetic influences on gene expression are a key source of variation in organismal traits. Many of these effects specifically alter protein abundance but not mRNA levels. The molecular basis of protein-specific effects remains mostly unknown. Protein degradation is a

promising potential target of DNA variants that influence proteins specifically, but genetic variation in protein degradation has not been studied systematically.

To address this shortcoming, we mapped genetic influences on the ubiquitin-proteasome system (UPS), the cell's primary pathway for targeted protein degradation. We combined 1) a statistically powerful method for mapping quantitative trait loci (QTLs) based on pooled whole-genome sequencing of large cell populations with extreme phenotypes in the yeast *Saccharomyces cerevisiae*, with 2) tandem fluorescent timers, molecular reporters that enable quantitative readouts of protein degradation in millions of single cells. We mapped variation in the degradation of a set of substrates that assay all 20 amino acids that act as degradation signals in the well-known UPS N-end rule, as well as two ubiquitin-independent substrates. We identified 167 UPS activity QTLs. Most QTLs were specific to individual UPS pathways or substrates, revealing a highly complex genetic basis of variation in UPS activity.

We used CRISPR-Cas9 genome engineering to resolve four QTLs to their causal genes and nucleotides. UPS activity was shaped by both regulatory and missense variants in ubiquitin system genes whose products process (*NTA1*), recognize (*UBR1*, *DOA10*), and ubiquitinate (*UBC6*) substrate proteins. Each of these genes carried multiple causal variants. The effects of individual causal variants were strikingly specific to distinct UPS pathways, including cases in which different variants in the same gene affected different UPS substrates. Evolutionary and population genetic analysis showed that causal variants that decrease UPS activity tend to be derived and at low population frequency, suggesting that they reduce organismal fitness.

To understand how causal variants for UPS activity influence gene expression, we tested the effect of a *cis*-acting causal variant in the *UBR1* promoter on genome-wide protein and RNA levels. The variant altered the abundance of 36 proteins without affecting levels of the corresponding mRNA transcripts, implicating genetic influences on the UPS as a prominent source of protein-specific effects on gene expression.

Our results reveal a complex genetic architecture of UPS activity, demonstrate how variation in ubiquitin system genes influences UPS protein degradation, and establish a framework for understanding how genetic effects on the UPS contribute to variation in cellular and organismal traits.

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An orthologous gene coevolution network provides insight into eukaryotic cellular and genomic structure and function

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Orthologous gene coevolution—which refers to gene pairs whose evolutionary rates covary across speciation events—is often observed among functionally related genes. We present a comprehensive gene coevolution network inferred from the examination of nearly three million orthologous gene pairs from 332 budding yeast species spanning ~400 million years of eukaryotic evolution. Modules within the network provide insight into cellular and genomic structure and function, such as genes functioning in distinct cellular compartments and DNA replication. Examination of the phenotypic impact of network perturbation across 14 environmental conditions using deletion mutant data from the baker's yeast *Saccharomyces cerevisiae* suggests that fitness in diverse environments is impacted by orthologous gene neighborhood and connectivity. By mapping the network onto the chromosomes of *S. cerevisiae* and the opportunistic human pathogen *Candida albicans*, which diverged ~235 million years ago, we discovered that coevolving orthologous genes are not clustered in either species; rather, they are most often located on different chromosomes or far apart on the same chromosome. The budding yeast coevolution network captures the hierarchy of eukaryotic cellular structure and function, provides a roadmap for genotype-to-phenotype discovery, and portrays the genome as an extensively linked ensemble of genes.

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Proteomic and phosphoproteomic analysis of the cellular response to mis-made proteins caused by mistranslating tRNA variants

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Life does not require a perfectly accurate proteome. Mistranslation, or the mis-incorporation of an amino acid that differs from what is specified by the genetic code, naturally occurs at a rate of one mis-incorporated amino acid in every 10^4 to 10^5 codons. Mistranslation rates increase in response to environmental conditions or due to mutations in the translational machinery. Cells have mechanisms to cope with mis-made proteins, allowing them to tolerate up to 10% mistranslation. In this work, we use a proteomics approach to investigate how cells respond to different mistranslation substitutions. We engineer yeast strains expressing tRNA variants that mistranslate either alanine at proline codons, serine at proline codons or serine at alanine codons. As excessive mistranslation prevents growth, we use a doxycycline inducible system to control tRNA expression and achieve low (~2.5%) and high (~5%) mistranslation frequencies for each substitution. Using whole proteome mass spectrometry, we identify both common and unique sets of differentially abundant proteins in response to the three substitutions. We also analyze the phosphoproteome, as phospho-signaling pathways can mediate the proteotoxic stress response, and identify differentially abundant phosphorylation sites in each mistranslating strain. In addition, two of the tRNA variants mis-incorporate serine and potentially introduce new phosphorylation sites that could impact protein function. Supporting this, we identify mistranslated serine residues that are phosphorylated. Overall, our results highlight the proteomic response to mistranslation, including the pathways and signaling responses cells use to cope with increases in translational errors. Of note, human genomes contain similar tRNA variants with the potential to mistranslate. The combination of these variants with disruptions in the pathways necessary for cells to cope with mis-made protein could contribute to disease.

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Decoupling growth and metabolism via PKA hyperactivation is linked to alterations in lipid homeostasis

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All organisms have evolved elaborate physiological processes that coordinate growth, proliferation, metabolism, and stress defense in response to ever-changing environments. While individual regulatory pathways have been well studied individually, how they integrate to produce global and systemic changes remains unclear. Past work from our lab has studied the coordination of growth, metabolism, and stress defense, focusing on *Saccharomyces cerevisiae* strains engineered to anaerobically ferment the non-native pentose sugar xylose. We discovered that network rewiring leading to hyperactivation of the RAS/Protein Kinase A (PKA) pathway is needed for rapid anaerobic xylose fermentation; however, the mechanism of PKA hyperactivation has a dramatic impact on cellular coordination: deletion of the RAS inhibitor *IRA2* permits rapid growth and fermentation, while deletion of the PKA regulatory subunit *BCY1* allows for vigorous fermentation without growth. To understand how *BCY1* deletion decouples growth and metabolism, we performed a multi-omic approach that integrated transcriptomic, metabolomic, and lipidomic analysis across strains. Integrating results pointed to significant differences in the expression and function of lipid metabolism and signatures of its effects. Remarkably, we discovered that growth and metabolism could be recoupled through directed evolution of the *bcy1*Δ strain to grow anaerobically on xylose, leading to point mutations in the PKA catalytic subunit *TPK1*, *OPI1* that encodes a transcriptional regulator of phospholipid genes, and *RIM8* which is important for anaerobic growth in a lab strain. Interestingly, Opi1 is the inhibitor of genes whose expression was altered in the *bcy1*Δ strain growing on xylose, including targets of the phospholipid transcriptional activator Ino4. Furthermore, the *bcy1*Δ strain accumulates various species of phosphatidylethanolamine when grown on xylose, suggesting a bottleneck in phospholipid metabolism directly linked to the functions of Opi1 and Ino4. Using this unique model, our work is contributing new insights into mechanisms of growth control and cellular coordination.

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Humanized yeast to measure the functional impact of human genetic variation in the mevalonate kinase gene

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Advances in sequencing technology have allowed the study of human genetic variation. Thousands of human genomes are currently available, identifying several thousand variants. A grand challenge is to determine what, if any, impact each missense mutation has on human health. The fact that many human genes can functionally replace their corresponding yeast equivalents provides an opportunity to use humanized yeast to model human genetic variation [1,2]. By swapping essential yeast genes with human counterparts, we can link the fitness of the variant human protein with the fitness of the yeast cell. Therefore, mutations that have functional consequences will show phenotypic differences in the humanized yeast, such as slower growth rate. In addition, our system allows for tunable control of gene expression levels, differing from studies that look only at complementation through overexpression.We demonstrate this approach on the rare human genetic disorder known as mevalonate kinase deficiency by devising a strategy to conditionally replace yeast Sc*ERG12* gene with the orthologous human mevalonate kinase gene, Hs-*MVK.* Next, we show the yeast growth as an easily measured proxy for the proper functioning of the human gene. By humanizing yeast with gene variants followed by sequencing as a readout to deconvolute each strain's relative growth, we can score the functional impact of human gene variation. Thus, neutral variants are easily distinguished from deleterious variants. This work, in addition to previous similar strategies [3], establishes a platform for using humanized yeast to model human genetic variation at scale. Through the application of a deep mutational scanning approach, we hope to greatly contribute to the current data on mevalonate kinase mutations. And as a result, further our understanding of mevalonate kinase deficiency.

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From Chemostats to Single Cells: Miniaturized Technologies for Monitoring Yeast Metabolic Cycles

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Budding yeast growing in glucose-limited chemostats will exhibit robust and synchronous oscillations in oxygen consumption. This phenomenon, called the yeast metabolic cycle (YMC), is accompanied by the periodic expression of genes and metabolites, which interact with, but can be independent of, the cell division cycle (CDC). Current tools for studying the YMC involve large, expensive bioreactors and manual extraction of samples for downstream analysis with biochemical or genomic assays. These bulk assays are population averages and do not provide any insights into metabolic and cell cycle dynamics within a single cell. To overcome these limitations, we developed two technologies.

The first is a cost-effective miniature (20-mL) bioreactor array. Its manageable size increases throughput, and autonomous computer control permits continuous, real-time fluorescence and luminescence measurements of multiple strains. By measuring metabolic states (i.e., NAD(P)H or trehalose) or cell cycle events (i.e., Cln3 translation or Clb2 transcription) within these small reactors, we show how key

metabolic and cell-cycle processes are coordinated relative to dissolved oxygen consumption. Surprisingly, we find that S288c and CEN.PK (the two most commonly used strains for the YMC) exhibit anti-phase timing of key processes relative to oxygen consumption.

This bioreactor array, although useful, is still limited to measuring population-averaged signals. This inspired our second technology, the Crypt Chip, a microfluidic device that permits the fluorescent imaging of cell cycle and metabolic reporters for trapped single cells that are responding to the real-time, continuous flow of chemostat-conditioned media. We show that mother cells commit to the CDC each YMC, whereas daughter cells have a prolonged G1 phase and commit to the CDC after a few metabolic cycles. Furthermore, both mothers and daughters exhibit a pulse of metabolic activity each YMC, such that many daughters undergo multiple pulses of metabolic activity before committing to the CDC. This work is important because it shows that the YMC occurs in single cells and that it can function independently of the CDC.

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Cross-talk between the DNA damage response and the Mediator of transcription

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The Mediator of transcription is a multi-subunit protein complex that is a critical component of RNA polymerase II-mediated transcriptional machinery which regulates the transcription of essentially all genes. The core Mediator contains a head, middle and tail module, and an accessory kinase module (CKM) which can reversibly associate with the core Mediator. CKM-bound core Mediator has generally an antagonizing effect on transcription; however, it can selectively to promote the transcription of specific transcripts in certain contexts. In addition to its opposing effects on transcription, CKM influences a variety of cellular processes including stress responses, genome organization and tumorigenesis. However, it is not well-understood how CKM incorporates signals from these divergent pathways, and whether these functions of CKM are dependent on its kinase activity and its interactions with core Mediator. Through a genome wide unbiased interaction screen in yeast to identify novel pathways that contribute to the regulation of DNA damage response, an intricate signaling pathway involved in protecting the integrity of the genome, we discovered a genetic interaction with the Mediator. Through a focused mutational screen of Mediator-CKM subunits in yeast, we found that all of the four CKM subunits as well as CKM's kinase activity are essential for cell cycle re-entry following a DNA break. Notably, the CKM mutants did not impair UV damage repair or DNA double-strand break repair through homologous recombination, indicating that CKM specifically impinges on DNA damage response. Furthermore, we demonstrated that CKM contributes to the global downregulation of transcription following DNA damage. Taken together, these findings reveal the molecular mechanisms of previously uncharacterized link between DNA damage and CKM.

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Cip1 tunes cell cycle arrest duration upon calcineurin activation

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Cells exposed to environmental stress arrest the cell cycle until they have adapted to their new environment. Cells adjust the length of the arrest for each unique stressor, but how they do this is not known. Here, we investigate the role of the stress-activated phosphatase calcineurin (CN) in controlling cell cycle arrest in *Saccharomyces cerevisiae*. We find that CN controls arrest duration through activation of the G1 cyclin-dependent kinase inhibitor Cip1. Our results demonstrate that multiple stressors trigger a G1/S arrest through Hog1-dependent downregulation of G1 cyclin transcription. When a stressor also activates CN, this arrest is lengthened as CN prolongs Hog1-dependent multisite phosphorylation of Cip1. Cip1 plays no role in response to stressors that activate Hog1 but not CN. These findings illustrate how stress response pathways cooperate to tailor the stress response and suggest that Cip1 functions to prolong cell cycle arrest when a cell requires additional time for adaptation.

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Decoding multisite phosphorylation by CDK

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Ordered and timely progression through the cell cycle depends on the phosphorylation of hundreds of proteins by cyclin dependent kinases (CDKs). CDK substrates are often phosphorylated at multiple sites that cluster in intrinsically disordered regions (IDRs). However, for many substrates, it remains unknown if phosphorylation at many sites or only key sites is required to regulate protein function. To address this question, we developed an *in vivo* selection assay, called Phosphosite Scanning, to comprehensively evaluate the contribution of each phosphosite to the regulation of a model CDK substrate. We applied this technique to the *S. cerevisiae* S phase transcription factor Hcm1. Phosphorylation across a cluster of eight sites in the C-terminal transactivation domain activates Hcm1 and influences cellular fitness. We leveraged this phenotype to screen a library of Hcm1 phosphomutants in which each CDK site has been mutated to unphosphorylatable or phosphomimetic residues in all possible combinations. Fitness measurements were then validated by testing the ability of mutants to complement loss of Hcm1 function *in vivo* and to activate transcription in a reporter assay. We found that Hcm1 activity is tunable and generally increases with the number of phosphomimetic mutations; however, particular sites are more potent. Two sites (T460 and S471) contributed the greatest amount to Hcm1 activation and were sufficient, though not required, to restore wild type activity. Screening of additional libraries that incorporate wild type sites revealed that the presence of upstream threonine residues was required to facilitate the phosphorylation of T460 and S471, suggesting that N-terminal threonine residues function as docking sites for the CDK processivity factor

Cks1. These results demonstrate that Phosphosite Scanning can be applied to understand the contributions of individual sites within a multiphosphorylated domain to the regulation of protein function.

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Quiescent yeast cells are equipped with the regulatory mechanism that suppresses re-entry into mitosis

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Cells of the budding yeast *Saccharomyces cerevisiae* form spores or stationary cells upon nutrient starvation. These quiescent cells are known to resume mitotic growth in response to nutrient signals but the mechanism remains elusive. Here, we report that quiescent yeast cells are equipped with a negative regulatory mechanism which suppresses the commencement of mitotic growth. The regulatory process involves a glycolytic enzyme, triosephosphate isomerase (Tpi1) and its product glyceraldehyde-3-phosphate (GAP). GAP serves as an inhibitory signaling molecule; indeed, the return-to-growth of spores or stationary cells is suppressed by the addition of GAP even in nutrient-rich growth media, though mitotic cells are not affected. Reciprocally, dormancy is abolished by heat treatment because of the heat sensitivity of Tpi1. For example, spores commence germination merely upon heat treatment, which indicates that the negative regulatory mechanism is actively required for spores to prevent premature germination. These results suggest that, in quiescent cells, nutrient signals do not merely provoke a positive regulatory process to commence mitotic growth. Exit from the quiescent state in yeast cells is regulated by balancing between the positive and negative signaling pathways. Identifying the negative regulatory pathway would provide new insight into the regulation of the transition from the quiescent to the mitotic state. Given that the quiescent state is modulated by manipulation of the negative regulatory mechanism, understanding this process is important not only for its biological interest but also as a potential target for anti-fungal treatment.

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A novel role for phosphatidylinositol-3,5-bisphosphate in mitosis

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Phosphoinositides, conserved from bacteria to human, are signaling lipids that recruit their effector proteins to specific cellular membrane locations, thereby providing instantaneous and local signaling. Among seven phosphoinositides, the least understood phosphoinositide is the Phosphatidylinositol-3,5-bisphosphate (PI3,5P2). PI3,5P2 is synthesized at the vacuole membrane and at very minute levels compared to other phosphoinositides. Cellular levels of PI3,5P2 increases in response to stress conditions such as hyperosmotic shock. PI3,5P2 has roles in stress response, membrane trafficking, vacuole/endolysosome structure/function and transcriptional regulation. No function has been attributed to PI3,5P2 in mitosis so far. In this study, we report a novel function for PI3,5P2 in the mitotic exit stage of the cell division cycle in budding yeast. Accordingly, lack of PI3,5P2 caused a delay in mitotic exit, whereas elevated levels of PI3,5P2 accelerated mitotic exit in mitotic exit defective cells. Using a known PI3,5P2 effector as a sensor we monitored PI3,5P2 in an unperturbed cell cycle.Through epistasis analysis we mapped PI3,5P2 pathway with respect to the known mitotic exit related pathways. Our data suggests a mitotic exit promoting role for PI3,5P2.

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Genetic regulation of genome stability in *Saccharomyces cerevisiae*

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Although genetic diversity is often assumed to be generated by meiotic recombination and segregation, genomic alterations that occur in mitotic cells are also a potent source of diversity. I will discuss the rate of genomic alterations (point mutations, large deletions/duplications, mitotic recombination events, and ploidy changes) observed in wild-type yeast diploids. The evidence that most observed mitotic crossovers are the result of double-stranded DNA breaks (DSBs) that occur in G_1 of the cell cycle will be described. The rate of genomic changes is substantially increased in yeast strains in which the level of the replicative DNA polymerases alpha, delta, or epsilon is reduced. Mitotic recombination events in strains with low levels of DNA polymerase occur as a result of DNA breakage in the S-period. By expressing the human protein APOBEC3B (a single-strand-specific cytosine deaminase), we show that strains with low levels of the replicative DNA polymerases have extensive regions of single-stranded DNA that likely represent preferred sites for DNA breaks.

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S. pombe wtf genes use dual transcriptional regulation and selective protein exclusion from spores to cause meiotic drive

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Meiotic drivers bias gametogenesis to ensure their transmission into more than half the offspring of a heterozygote.
In *Schizosaccharomyces pombe*, *wtf* meiotic drivers destroy the meiotic products (spores) that do not inherit the driver from a heterozygote, thereby reducing fertility. *wtf* drivers encode both a Wtf^{poison} protein and a $Wtf^{antidote}$ protein using alternative transcriptional start sites. Here, we analyze how the expression and localization of the Wtf proteins are regulated to achieve drive. We show that transcriptional timing and selective protein exclusion from developing spores ensure that all spores are exposed to $Wtf4^{poison}$, but only the spores that inherit *wtf4* receive a dose of $Wtf4^{antidote}$ sufficient for survival. In addition, we show that the Mei4 transcription factor, a master regulator of meiosis, controls the expression of the *wtf4*^{poison} transcript. This transcriptional regulation, which includes the use of a critical meiotic

transcription factor, likely complicates the universal suppression of *wtf* genes without concomitantly disrupting spore viability. We propose that these features contribute to the evolutionary success of the *wtf* drivers.

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Species-wide exploration of the inherited gene expression variation in yeast

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Gene expression regulation is an essential step in the translation of genotypes into phenotypes. However, the transcriptional landscape as well as the genetic effects on gene expression variation are poorly characterized at a species-wide level. By taking advantage of the completely sequenced set of 1,011 *Saccharomyces cerevisiae* yeast isolates, we present here a deep analysis of RNA sequencing data across this large population. The exploration of the pan-transcriptome, composed of 4,936 core and 1,734 accessory genes highlighted a differential transcriptional behavior between these two sets of genes. We also found clear transcriptional signatures related to different domestication processes by leveraging the large diversity of subpopulations. In addition, we comprehensively characterized genetic associations for gene expression, showing that CNV-eQTL explained a larger fraction of the gene expression variance compared to SNP-eQTL. Moreover, accessory genes are proportionally associated to a larger number of eQTL with a higher variance explained. Overall, these findings illustrate how the accessory genome largely contribute to genetic effects on gene expression and represent a key component, shaping the transcriptional landscape at the subpopulation level.

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Evolution of Acquired Resistance for Hydrogen Peroxide Involves Differential Sensing of Phosphate by TORC1 and Activation of the Transcriptional Factor Msn4 in *C. glabrata* compared with *S. cerevisiae*

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Environmental stresses often occur in combination or in close succession. Thus, the ability to anticipate and prepare for upcoming stresses likely provides an important fitness advantage. We found that *Candida glabrata*, an opportunistic yeast pathogen belonging to the sister genus of *Saccharomyces* that contains *S. cerevisiae*, exhibits acquired stress resistance (ASR) for severe hydrogen peroxide (H_2O_2) challenge after being exposed to a period of phosphate starvation. Similar protection in *S. cerevisiae* is much weaker and requires longer treatment of the primary stress. Transcriptome analysis revealed that phosphate starvation induced a number of oxidative stress responsive genes in *C. glabrata* but not in *S. cerevisiae*. These include the only catalase in *C. glabrata* encoded by *CTA1*. *cta1*Δ not only renders *C. glabrata* cells more sensitive to H_2O_2, but also abolishes the phosphate starvation-induced ASR effect. Among known transcription factors regulating stress responses, we found the general stress response transcription factor Msn4 is a major activator required for *CTA1* induction during phosphate starvation. PKA and TORC1, two conserved and central nutrient-sensing kinase complexes, negatively regulate Msn4. We found TORC1 is rapidly inactivated during phosphate starvation in *C. glabrata* but not in *S. cerevisiae*. *CTA1* induction in *C. glabrata* during phosphate starvation is abolished by genetically activating PKA or deleting Rim15, a kinase negatively regulated by both PKA and TORC1. Together, our results demonstrate that organisms' ability to predict upcoming stress is an evolvable trait that involves modifying the activity of conserved nutrient and stress-sensing regulators such as TORC1, PKA and Msn4.

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Kfc1, the yeast ortholog of Virilizer, is required for mRNA m^6A methylation and meiosis

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N^6-Methyladenosine (m^6A) is one of the most abundant modifications made to eukaryotic mRNAs. The m^6A modification has varying effects on the fate of transcripts as it helps coordinate the regulation of a host of complex biological processes, including meiosis. Meiosis is a specialized cell division program that results in the formation of cells (gametes) that convey genetic material between organisms and across generations. During budding yeast meiosis, m^6A levels peak early, before the initiation of the meiotic divisions. Ygl036w is a previously uncharacterized protein with no discernible domains except for several intrinsically disordered regions. Recent advances in protein folding prediction tools, showed that Ygl036w has several conserved motifs and is predicted to fold like *Drosophila* Virilizer, which is involved in mRNA methylation. High-throughput studies suggested, and work from our lab confirmed that Ygl036w interacts with meiotic proteins involved in mRNA methylation (Kar4, Mum2, Ime4, and Slz1) supporting a role in meiosis and mRNA methylation. Our lab identified alleles of Kar4 that are specifically defective for either of its two meiotic functions (Mei- alleles, defective for transcriptional induction and suppressed by over-expression of the transcription factor Ime1, and Spo- alleles, blocked later in meiosis and requiring co-suppression by Rim4). We found that the Spo- Kar4 mutants are specifically defective for interaction with Ygl036w. Accordingly, we propose to call the gene *KFC1* for *KAR4 Collaborator 1*. Kfc1 is essential for meiosis and *kfc1*Δ mutants have a severe loss of mRNA methylation. Moreover, Kfc1 is required for the stability of Kar4, Mum2, and Ime4, suggesting that Kfc1 may act as a scaffold to stabilize the methyltransferase complex. Like *kar4*Δ, the *kfc1*Δ mutant is defective for premeiotic S-phase, which is suppressed by over-expression of *IME1;* additional over-expression of the translational regulator *RIM4* is required for sporulation. Consistent with suppression by *IME1* over-expression, kfc*1*Δ has an early meiotic defect in the abundance of transcripts within Ime1's regulon (as well as *IME1* itself). A later defect in the expression of the middle meiotic transcription factor, Ndt80 (and genes in its regulon), is rescued by over-expressing both *IME1* and *RIM4.* Together, these data suggest that Kfc1 is required for cells to initiate the meiotic program, possibly through *IME1,* and for progression through meiosis and spore formation.

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Functional dissection of the RNA polymerase trigger loop by deep mutational scanning

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Eukaryotic multisubunit RNA Polymerases (Pol) are conserved from yeast to human. We have dissected relationships among polymerase residues and how epistasis affects evolution within eukaryotic RNA polymerases by employing a deep mutational scanning “structural genetics” approach in yeast. RNA Pol I, II, III are structurally conserved though they have evolved to have their own regulation and produce different classes of transcripts. At the heart of these RNA polymerases is an ultra-conserved active site domain, the trigger loop (TL), that participates in transcription by switching between an open, catalytic-disfavoring state and a closed, catalytic-favoring state. Mutations in the Pol II TL residues affect substrate selection, catalysis and translocation, suggesting the TL residues are critical to every aspect of transcription elongation. Additionally, the TL’s function is shaped by interactions with adjacent protein domains. Our lab has observed complex functional residue-residue interactions in Pol II TL, indicating transcription activity is facilitated by a functional network within the TL and between the TL and adjacent domains. Furthermore, previous studies have found that identical mutations in a residue conserved between the Pol I and Pol II TLs yielded different biochemical phenotypes, implying even functions of conserved residues may be shaped by individually evolved enzymatic contexts (epistasis). In order to understand TL residue interactions that shape polymerase mechanisms, we have functionally dissected Pol II TL mutants and intra-molecular genetic interactions in a high throughput genetic phenotyping system. Through analysis of over 14000 alleles, representing single mutants, a subset of double mutants, and evolutionarily observed TL haplotypes, we have identified residue interactions within the TL and between TL and adjacent domains. Moreover, we are investigating functional residue coupling across evolution and identifying where epistasis within Pol II constraints TL residue identity and function. We are also extending this system to Pol I and III to comprehensively compare and contrast residue requirements across polymerase evolution. Our studies provide a powerful comparative system to understand the plasticity of RNA polymerase mechanisms.

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The anticodon determines the impact of mistranslating tRNA^{Ala} variants in *Saccharomyces cerevisiae*

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Transfer RNAs (tRNAs) maintain translational fidelity through strict charging by their cognate aminoacyl-tRNA synthetase (aaRS) and through codon:anticodon base pairing with the mRNA at the ribosome. Since alanyl-tRNA synthetase uniquely recognizes a G3:U70 base pair in the acceptor stem of tRNA^{Ala} and the anticodon plays no role in charging, tRNA^{Ala} variants with anticodon mutations can give rise to mis-incorporation of alanine at non-alanine codons, a process known as mistranslation. Our goal was to characterize the phenotypic consequence of expressing all 60 tRNA^{Ala} anticodon variants in *S. cerevisiae*. To control mistranslation-associated toxicity, we used a tetracycline inducible system to regulate tRNA expression. Overall, 36 tRNA^{Ala} anticodon variants decreased growth when expressed in single-copy or multi-copy, representing 15 of the 19 possible substitutions to alanine. Surprisingly, variants decoding synonymous codons, such as leucine, threonine and valine anticodon variant families, showed a wide range of impacts on yeast growth, despite making the same amino acid substitution. We considered several factors, such as codon abundance, buffering by native yeast tRNAs and expanded wobble decoding to explain these differences. In particular, decreased growth was observed for variants with GC rich anticodons compared to AT counterparts decoding synonymous codons, suggesting increased decoding strength by G:C base pairs. We used mass spectrometry to measure the frequency of alanine mis-incorporation events, which correlated well with observed growth impacts. Furthermore, variants lacking growth effects in a wild-type background resulted in slow growth when the anticodon-cognate synthetase was knocked down. This suggested that charging or cross-editing by the cognate synthetase may limit the mistranslation potential of some variants. In addition to highlighting novel tRNA biology, these variants have applications in synthetic biology for generating pools of statistical proteins with novel functions. Since potential mistranslating tRNA variants also exist in human populations, our analysis exposes tRNA variants that may exacerbate disease.

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72A

rDNA copy number is a determinant of replicative lifespan and facilitates further dissection of the aging processes in yeast.

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The genetic and environmental determinants of lifespan are not well understood for any organism. Recent technological advances have made it possible to study these effects in a relatively high-throughput and systematic way in budding yeast. Microfluidic technologies allow longitudinal observations of the entire lifespan of single yeast cells and improvements in culturing and purification strategies allow preparation of large populations of aged cells. Using these tools, we recently reported on a previously unappreciated relationship between chromosomal ribosomal DNA copy number (rDNA CN) and replicative lifespan (RLS) [Hotz M, Thayer NH, Hendrickson DG, et al. PNAS 2022]. Through a previously established relationship between rDNA CN and the levels of extrachromosomal rDNA circles (ERCs), a well-characterized aging factor, variations in rDNA CN number explained over 70% of the observed variation in the RLS of a panel of otherwise wild-type strains. Because rDNA CN is known to spontaneously vary and be influenced by both genetic and environmental factors [Kwan EX et al. G3 2016; Puddu F, et al. Nature 2019; Mansisidor A, et al, Mol. Cell 2018], rDNA CN (and linked aging pathways) must be considered when evaluating lifespan and aging in yeast.

Here, we will present our work using the relationship between rDNA CN and lifespan to further dissect aging pathways and trajectories: i) by analyzing single cells and the phenotypes they experience ii) by evaluating lifespan in various rDNA CN contexts iii) and employing a genome-wide screen for lifespan in an rDNA CN insensitive background. These findings identify other phenomena unrelated to rDNA that affect aging, and how different aging trajectories relate to one another.

73A

Variability of the probiotic yeast *Saccharomyces cerevisiae* var. *'boulardii'* in genome, phenotype, and virulence

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Saccharomyces yeast probiotics (*S. 'boulardii'*) belong to the Wine/European clade of *S. cerevisiae*. They have long been successfully applied in the treatment of several gastrointestinal conditions, including *C. difficile* infection and diarrhea. However, these products sometimes cause fungaemia in susceptible patients, hence research on the pathomechanism and adaptive properties of probiotic yeast is important. The potential virulence attributes of *S. 'boulardii'* as well as its interactions with the human immune system have thus been in the focus of various studies, most often focusing on a single or very few product isolates, thereby neglecting the possible variability of strains and isolates.

Here, we aimed to study how different various product and human isolates (from infection/colonization events) of the probiotic yeast may be in their genomes and genome structure, their phenotype, virulence attributes, immunological interactions, and pathogenicity (in BALB/c immunosuppressed mouse model). We also exploited CRISPR/Cas9 technology to conduct gene deletions to determine the effects of potential virulence genes, including the gene heme oxygenase-1 (*HMX1*) involved in iron recycling and utilization. Gene deletion was carried out in six various genetic backgrounds (both commercial and human isolates).

Our results showed that both human and product isolates are variable in terms of loss-of-heterozygosity regions and SNPs affecting gene functions in various cellular processes, and this variability is even evident among batches of the same product. In-host selection was not found to be directed towards higher expression of virulence factors. Furthermore, the deletion of the *HMX1* gene significantly increased the survival of strains in the fungemia mouse model instead of decreasing it, as presumed. The gene deletion had variable effects overall on the *in vitro* phenotypes of the six applied *S. 'boulardii'* genetic backgrounds, highlighting the fact that even closely related yeasts may respond differently to loss-of-function mutations and to genetic manipulation.

As genetically modified probiotic and biotherapeutic organisms are on the rise, our results call attention on the fact that not only are the various probiotic yeasts different in many aspects, but editing their genomes may bring forth unexpected effects as well.

74A

Engineering all-substitution gene variant libraries using a single Cas9 guide RNA target sequence in pooled yeast populations

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Thorough study of genetic diseases requires understanding how amino acid substitutions influence phenotype. Manually engineering individual substitutions can provide insight into small numbers of variants, but assessing all possible variants requires high-throughput methods. Here, we describe a method that can generate and evaluate all possible single amino acid substitutions for any gene of interest, so long as the gene exhibits selectable influence on cell growth or survival of Saccharomyces cerevisiae, including both native yeast genes their human orthologs. Importantly, our method improves upon deep mutational scanning by allowing for high-throughput selection, sequencing, and phenotyping of variants within a single pool, using a single shared Cas9 guide RNA target sequence. We assemble a variant-generating plasmid library containing tens of thousands of specific repair templates from a synthesized oligonucleotide array. Each plasmid, when transformed into a yeast cell expressing Cas9, is designed to induce a specific amino acid substitution. We transform our plasmid library into a yeast strain that possesses two copies of our gene of interest separated by a counter-selectable marker. The repair templates induce large-scale deletions between the gene copies, removing the counter-selectable marker while introducing a novel codon. This deletion-based method greatly simplifies CRISPR-Cas9 editing by using a single, shared guide RNA for the entire variant library. This method facilitates pooled tracking of variant abundance (inferred from abundance of repair templates in pooled short-read sequencing) for rapid evaluation of variant effects on protein function.

75A

Impact of environmental stress on self-perpetuating protein aggregation in yeast

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Cross-beta fibrous protein aggregates, termed amyloids, are associated with devastating human and animal diseases. Self-perpetuating protein isoforms, termed prions and typically (but not always) based on amyloids, are capable of transmitting infectious diseases and/or carrying heritable information. Yeast prions control heritable traits, manifesting themselves as a valuable model for studying cellular modulation of transmissible protein aggregates. Metastable prions and non-transmissible aggregates (mnemons) maintain cellular memory of environmental or physiological changes. Environmental stresses lead to massive accumulation of aggregated proteins. Toxic effects of stress-induced protein aggregation are counteracted by the chaperone machinery. Ribosome-associated chaperone Hsp70-Ssb has previously been shown by us and others to counteract formation and propagation of the prion form of yeast translation termination factor Sup35. Here, we demonstrate that Hsp70-Ssb also antagonizes formation and/or heritability of a variety of self-perpetuating aggregates generated by other yeast proteins. For example, heat stress results in the accumulation of prion form of the actin-associated protein Lsb2 in up to 20% of the cells lacking Hsp70-Ssb. This implicates Hsp70-Ssb as a major regulator of the stress-induced heritable protein aggregation. We also uncover the impact of environmental stress on the formation of non-heritable liquid-liquid biocondensates by the constructs bearing the prion domain of the yeast Sup35 protein. Previously, biocondensate formation by Sup35 was observed in response to a decrease of pH, however our data show that Sup35 biocondensates are also promoted by osmotic stress, that is not associated with acidic pH. The evolutionary conservation of the biocondensate formation in the response to osmotic stress by Sup35 proteins from different yeast species is investigated, and a relationship between biocondensate formation and generation of heritable (prion) aggregates by Sup35 is addressed. An impact of stress on self-perpetuating protein aggregation, detected in yeast, could be relevant to human amyloid diseases.
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76A

Glycan remodeling on the yeast surface to permit directed evolution of antibody Fc regions

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Antibodies are critical for a functional immune system. The mechanism of action of antibodies is divided into two distinct functions performed by two distinct regions of the molecule – recognition by fragment antigen-binding (Fab) regions and signaling by fragment crystallizable (Fc) regions. Fc-dependent signaling mechanisms, or antibody-mediated effector functions, occur through Fc interactions with Fc receptors on immune cells. The linkage of a complex biantennary glycan to a conserved *N*-linked glycosylation site on Asn297 allows these antibodies to efficiently interact with Fc γ receptors (FcγRs); antibodies missing this glycan exhibit markedly reduced effector functions. Because of the correlation between IgG-FcγR binding affinity and response to therapy, antibody engineering for increased binding affinity to FcγRs is being actively pursued. Yeast display is a common platform for performing directed evolution of proteins. However, yeast decorate their proteins with high-mannose glycans, which are incompatible with high-affinity FcγR binding. Chemoenzymatic synthesis methods to customize glycan chemistries on glycoproteins *in vitro* have been well developed. We have combined these methods and created an entirely new technology, Glycan Remodeling Yeast Display (GRYD) which permit directed evolution of properly glycosylated antibodies. We expressed IgG1 Fc region on the yeast surface. As were predicted, high-mannose glycosylation at Asn297 on Fc prevented binding of $FcγRIIIA_{V158}$, as measured by flow cytometry. However, binding was increased slightly when the yeast cells were treated with EndoA, an endoglycosidase active on high-mannose glycans. We observed a large increase in binding when the yeast cells were subsequently treated with $EndoS2_{D184M}$ and complex glycan oxazoline, an enzyme/substrate combination that efficiently transfer complex glycans to deglycosylated antibodies. When we expressed the Fc mutant N297Q, which cannot be *N*-linked glycosylated, we observed consistently low binding to $FcγRIIIA_{V158}$ by flow cytometry. We now produce and display a randomized library of Fc proteins on the yeast cell surface to select Fc variants with increased affinity to the common polymorphic variants of FcγRIIIA. Our GRYD technology will lead to creation of the next generation of immunotherapeutic antibodies with fully customizable antibody-mediated effector functions.

77A

The Canadian Rare Diseases Models and Mechanisms (RDMM) Network: Connecting novel disease gene discoveries to functional characterization research in model organisms

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Advances in genomics has transformed our ability to identify the molecular cause of rare diseases (RDs). Yet for most candidate RD genes, we lack insight into their biological function, how mutations identified in patients affect them, or what therapies could be useful. Model organisms (MOs) represent powerful tools to confirm the pathogenicity of RD gene variants, characterize gene's biological function, and identify potential therapies. For these reasons, the Canadian RDMM Network (http://www.rare-diseases-catalyst-network.ca/) was established in 2014 to catalyze and fund connections between clinicians discovering new disease genes and researchers able to study equivalent genes and pathways in MOs.

The RDMM Network has created a rapid and direct pathway from gene discovery to functional characterization studies in MOs. The central resource of the RDMM Network is a web-based Canadian directory of MO researchers ('the Registry') built to facilitate identifications of suitable collaborators for applying clinicians. As of May 2022, more than 650 MO researchers have registered over 16,000 genes of interest. With the aid of the computational inference built into the Registry, this translates to the coverage of more than 9,000 human genes. RDMM

uses a committee process to identify and review potential clinician-MO researcher matches and approve $25,000 CAD in catalyst funding. Since 2014, made 110 new clinician-MO scientist connections and funded 142 functional characterization proposals. Besides the scientific insights into the molecular mechanisms of rare disease and possible novel therapies, these collaborations also lead to high impact papers, long-term collaborations, external grants.

Leveraging on the success of the first four years, last year we expanded the impact and reach of the RDMM Network: we established international linkages with emerging similar networks in Europe, Australia, Japan and the United States. To facilitate further community uptake and adaptation of the concepts we established, we have made the RDMM Registry portable, customizable and linkable with other instances, and our committee structures and process freely available. (https://rdmminternational.org/)

The Canadian RDMM Network continues to expand its reach by adopting policies and processes to support global collaborations. We freely share our committee structures and processes, and are willing to assist emerging RDMM regional networks as needed. In so doing, we will continue to create meaningful collaborations between clinicians and MO researchers, generate new knowledge, and advance RD research locally and globally.

78A

Plant extract targetting α-synuclein mediated toxicity in Parkinson Disease models

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Neurodegenerative disorders are among the most prevalent human diseases affecting millions of people worldwide. The accumulation of amyloid aggregates is a characteristic feature of these diseases such as in case of Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD). Though these amyloids are composed of different proteins in different diseases, they share a hallmark core structure primarily consisting of β-sheet conformation. At cellular extent these diseases share common pathological hallmark such as proteasomal dysfunction, mitochondrial dysfunction, oxidative stress suggesting that the central process of cellular toxicity could be similar in numerous amyloid-based disorders.

In one of the ongoing study, we attempted to screen various plant extracts. Using yeast model we identified one of the extract that reduces α-synuclein mediated toxicity. In higher models such as *C.elegans* the extract tends to reduce α-synuclein aggregates as well as improves the body bending capability. It has also been seen that it helps to increase the longevity of *C.elegans* in control as well as transgenic α-synuclein strains. Having obtained these promising results, our future aim is to fractionate and identify the major compounds present in the crude extract that help to reduce α-synuclein mediated toxicity and to test this extract in higher eukaryotic models such as mice models of Parkinson's disease.

79A

The human pathogenic yeast *Cryptococcus neoformans* can utilize ferritin as an iron source

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Ferritin, a major iron storage protein in vertebrates, supplies iron upon iron deficiency. Ferritin is also found extracellularly, and acts as an iron carrier and a contributor to the immune response to invading microbes. Some microbial pathogens take advantage of ferritin as an iron source upon infection. However, no information is currently available on whether the human fungal pathogen *Cryptococcus neoformans* can acquire iron from ferritin. Here, we found that *C. neoformans* grew well in the presence of ferritin as a sole iron source. We showed that the binding of ferritin to the surface of *C. neoformans* is necessary and that acidification may contribute to ferritin-iron utilization by the fungus. Our data also revealed that the high-affinity reductive iron uptake system in *C. neoformans* is required for ferritin-iron acquisition. Furthermore, phagocytosis of *C. neoformans* by macrophages led to increased intracellular ferritin levels, suggesting that iron is sequestered by ferritin in infected macrophages. The increase in intracellular ferritin levels was reversed upon infection with a *C. neoformans* mutant deficient in the high-affinity reductive iron uptake system, indicating that this system plays a major role in iron acquisition in the phagocytosed *C. neoformans* in macrophages.

80B

tRNA synthetase inhibitors increase lifespan in a *GCN4* dependent manner

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Deletion of genes encoding ribosomal proteins extends replicative lifespan in the budding yeast, *Saccharomyces cerevisiae*. This increases translation of the functionally conserved transcription factor Gcn4, and lifespan extension in these mutants is largely *GCN4*-dependent. Gcn4 is also translationally upregulated by increased levels of uncharged tRNAs through Gcn2 activity. tRNA synthetase inhibitors are a class of drugs that prevent the charging of tRNAs resulting in an increase in uncharged tRNAs in the cell. We have shown that tRNA synthetase inhibitors upregulate Gcn4 translation and extend yeast lifespan in a *GCN4*-dependent manner. Gcn4 upregulation has been coupled with reduction in translation, however, we show that the reduction in global translation due to tRNA synthetase treatment is independent of Gcn4 activity. As Gcn4 is a transcription factor with many hundreds of targets, we aim to discover which transcriptional targets are responsible for the increased lifespan seen in strains with deletions of genes encoding ribosomal proteins and in yeast treated with tRNA synthetase inhibitors. We have performed RNA sequencing analysis on these long lived yeast and their normal lived *GCN4* deleted counterparts to determine what targets of Gcn4 are responsible for the lifespan extension.

81B

A structure-function method to identify pathogenic missense single nucleotide variants in *COQ5*, a gene encoding a *C*-methyltransferase in coenzyme Q biosynthesis

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The *COQ5* gene encodes an *S*-adenosylmethionine (AdoMet)-dependent enzyme responsible for catalyzing the *C*-methylation step in coenzyme Q (CoQ) biosynthesis in humans and in the yeast *Saccharomyces cerevisiae*. Coq5-deficient yeast lack CoQ and exhibit respiratory deficiency as a result of defective mitochondrial electron transport; they are also hypersensitive to treatment with polyunsaturated fatty acids due to their inability to use $CoQH_2$ as a chain-terminator of lipid peroxidation. Likewise, a defective CoQ biosynthetic pathway in humans can cause mitochondrial, cardiovascular, kidney, and neurodegenerative disorders through a condition known as primary CoQ deficiency. Here, we focus on human *COQ5* missense single nucleotide variants (SNVs) found in the NCBI ClinVar database, gnomAD, and Missense3D-DB, most of which have unknown clinical significance. Using multiple sequence alignments and available crystal structures of the yeast Coq5 polypeptide, we identify SNVs of potential clinical relevance in conserved methyltransferase motifs and functional regions of human COQ5. A comprehensive assessment of the structural and functional impact of the identified SNVs is facilitated by the Missense3D mutation classifier. Next, we will verify the pathogenicity of notable SNVs *in vitro* and *in vivo* to study their effect on catalytic activity, as well as their impact on CoQ biosynthesis and on the ability to assemble or stabilize the CoQ synthome, a high molecular mass complex required for CoQ biosynthesis. Collectively, our results will shed light on the structure-and-function relationship of the polypeptides encoded by yeast and human *COQ5* and facilitate the diagnosis of primary CoQ deficiency patients.

82B

Genetic Polymorphism that underlies Fungal Persistence interactions across a Mammalian Host

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Opportunistic fungal infections are a major public health threat with limited therapeutic options. The model budding yeast Saccharomyces cerevisiae can potentially be used to understand mechanisms enabling fungi to infect the human body, enabling new therapies. Numerous S. cerevisiae isolates have been obtained from clinical infections and the ability to infect humans does not appear universal among strains. To help understand how genetic factors give rise to opportunistic pathogenicity in S. cerevisiae, we previously injected barcoded segregants from across of a clinical isolate and a lab strain mice. Two classes of segregants were able to persist in the mouse body--one specialized on the brain and the other on non-brain organs--and genetic factors causing these differences were identified. Now, we plan to determine the biological significance of our past findings and to extend our work in key ways that maximally utilize this model for opportunistic fungal pathogenicity. Here, we provide a summary of this ongoing research.

83B

Identifying critical PxP adaptor binding sites in the yeast Vps13 VAB domain

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Lipids can be trafficked by lipid transport proteins at membrane contact sites (MCSs), protein-tethered contacts between adjacent organelles. Vacuolar protein sorting 13 (Vps13) and homologous proteins (e.g. Atg2) tether and, uniquely, directly transport lipids in bulk between membranes at MCSs.

The single yeast Vps13 homolog acts at MCSs at multiple organelles, including mitochondria, vacuoles, endosomes, and prospore membranes during sporulation. Organelle-specific adaptor proteins at these MCSs have a conserved proline-X-proline (PxP) motif that interacts with the Vps13 adaptor-binding (VAB) domain and targets Vps13 to these organelles.

The VAB domain consists of six repeats. Repeats 5 and 6 (R5 and R6) are sufficient to interact with PxP adaptors, while invariant asparagines in repeats 1 and 6 are necessary in the context of the full-length protein. Despite these findings, the exact binding interface between VAB and all PxP motifs has yet to be discovered. Using the novel protein modeling software, AlphaFold, and its derivative, ColabFold, we identified high confidence interactions between Vps13 and three well-established PxP motif-containing adaptors. From these models, the binding interface between Vps13 and its adaptors was predicted to involve two residues of VAB R5, at least six residues of VAB R6, and at least five hydrophobic residues of each PxP adaptor.

Mutagenesis was used to generate two vps13 alleles with mutations in either R5 or R6 that are predicted to disrupt VAB-PxP interactions based on ColabFold models. Several cellular phenotypic assays were conducted to assess the relative effects of these mutations. The R5 mutation yielded a mild reduction in the binding and localization of PxP adaptors, whereas the two R6 mutations significantly reduced interactions with all three PxP adaptors tested.

These findings support the AlphaFold predictions that show a single binding site located at the interface of VAB repeats 5 and 6 interacts with the PxP motifs of three well-established Vps13 PxP adaptor proteins.

84B

Protein interactions of PAS kinase, Pbp1, and Ptc6 in yeast reveal potential targets for ALS treatment

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Approximately 5000 people are diagnosed with Amyotrophic Lateral Sclerosis (ALS) every year. This fatal disease has no cure. 90 percent of cases occur sporadically, while 10 percent are familial, meaning the disease developed due to a genetic cause. Mutations in the Ataxin-2 gene have recently been shown to increase risk for ALS, making understanding the molecular pathways of Ataxin-2 activity of increasing interest. Our studies of the yeast homolog of ataxin-2, poly(A)-binding protein 1 (Pbp1), revealed that under glucose depravation, the nutrient sensing protein kinase PAS kinase phosphorylates and activates Pbp1. We have also identified 32 novel interaction partners of Pbp1. Most of these interacting partners are involved in RNA processing, while others are involved in DNA damage response, mitophagy, and misfolded protein response. Due to the metabolic alterations seen in ALS patients, we decided to investigate the interaction between Pbp1 and Ptc6, a protein involved in mitophagy. Since mitophagy is the clearing out of damaged mitochondria, and accumulation of defective mitochondria have been implicated in ALS, understanding these molecular pathways could prove important. A yeast-2-hybrid showed Ptc6 to be a direct binding partner of Pbp1. Our studies have found that in the absence of Pbp1, Ptc6 is hyperactive, and the cell has increased levels of mitophagy. Further studies revealed that the absence of Ptc6 increases both total mitochondria, as well as mitochondrial reactive oxidative species. In the absence of both Pbp1 and Ptc6, there is an increase in cellular respiration. By using a yeast model we have been able to set a foundation for further research into how the human homologs of these proteins are affecting mitochondrial regulation.

85B

Identifying Genes Required for Nuclear Rejuvenation during Gametogenesis

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In yeast, lifespan is reset during gametogenesis as senescence-associated factors are excluded from gametes through sequestration into a distinct nuclear envelope-bound compartment. Known as the Gametogenesis Uninherited Nuclear Compartment (GUNC), this compartment is subsequently degraded through a mega-autophagy event. Although instrumental in resetting lifespan, the mechanism by which age-induced damage is selectively sequestered into the GUNC remains unknown. To approach this question, we are developing a genetic screen that aims to identify factors involved in GUNC compartmentalization and selective nuclear inheritance. The screen will be conducted by targeting a plasmid containing a selectable marker to the GUNC and using the yeast deletion collection to identify genes required for the plasmid sequestration and subsequent elimination. To target the plasmid to the GUNC, we engineered a conditionally-excisable centromere, thereby mimicking an extrachromosomal circular DNA (ecDNA) from the rDNA locus that normally accumulates in aged cells, but is excluded from gametes. However, this alteration alone is not sufficient to mimic ecDNA. To improve plasmid exclusion from gametes, we fused aggregate-prone nucleolar proteins to the plasmid. Excitingly, one such fusion enhanced plasmid exclusion by 2-fold. In parallel, we employed a candidate approach to determine whether genes involved asymmetric segregation during mitosis are also required for exclusion of senescence-associated factors during gametogenesis. Together, these approaches offer a mechanistic understanding of how gametes are able to become devoid of age-induced nuclear defects.

86B

Analyses of missense single nucleotide variants in the *COQ4*, *COQ7*, and *COQ9* genes encoding proteins essential for coenzyme Q biosynthesis

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Coenzyme Q (CoQ) is a vital lipid that functions as an electron carrier in the mitochondrial electron transport chain and as a membrane-soluble antioxidant. The content of CoQ declines with age, and deficiencies in CoQ lead to metabolic diseases with a wide range of clinical manifestations. Currently, the only treatment available for such conditions is high-dose supplementation of CoQ_{10}. Mutations in any of the *COQ* genes responsible for CoQ biosynthesis may lead to a condition known as primary CoQ deficiency. While many mutations in these genes have been identified, the vast majority of the missense single nucleotide variants (SNVs) present in the NCBI ClinVar database, gnomAD, or Missense3D-DB have unknown clinical significance. Here we focus on classifying missense SNVs in genes *COQ4*, *COQ7*, and *COQ9*, all of which encode constituent proteins of a high molecular mass complex known as the CoQ synthome. *COQ4* encodes a putative lipid-binding scaffolding protein that organizes the CoQ synthome, and a complex of the polypeptides encoded by *COQ7* and *COQ9* catalyzes an aromatic ring-hydroxylation that comprises the penultimate step in CoQ biosynthesis. Using multiple sequence alignments and structural analyses on available models and crystal structures, we identify conserved and functional regions within each of these three genes. We utilize mutation classifiers Missense3D, SIFT, and PolyPhen2 to analyze the functional impact of known human missense SNVs together with identified conserved and functional regions of each sequence to identify those amino acid substitutions that are potentially pathogenic. These findings will serve as a basis for future experimental and biochemical characterizations of these SNVs to determine their functional impact on CoQ biosynthesis in yeast models. Collectively, our results will provide a resource for clinicians during patient diagnosis and guide therapeutic efforts toward combating primary CoQ deficiency.

87V

Impact of alanyl-tRNA synthetase editing deficiency in yeast

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Aminoacyl-tRNA synthetases are essential enzymes that provide the ribosome with aminoacyl-tRNA for protein synthesis. Mutations in aaRSs lead to various neurological disorders in humans. Many aaRSs utilize editing to prevent translation error during translation. Editing defects in alanyl-tRNA synthetase (AlaRS) cause neurodegeneration and cardioproteinopathy in mice and is associated with microcephaly in human patients. The cellular impact of AlaRS editing deficiency in eukaryotes remains unclear. Here we use yeast as a model organism to systematically investigate the physiological role of AlaRS editing. Our RNA sequencing and quantitative proteomics results reveal that AlaRS editing defects activate the general amino acid control pathway and attenuate the heatshock response. We have confirmed these results with reporter and growth assays. In addition, AlaRS editing defects downregulate carbon metabolism and attenuate protein synthesis. Supplying yeast cells with extra carbon source partially rescues the heat sensitivity caused by AlaRS editing deficiency. Our study therefore highlights the important role of AlaRS editing and provides a model for the physiological impact caused by the lack of AlaRS editing.

88V

Impairment of yeast PRPP synthetase activity affects cell signalling - a model for management and treatment for human neuropathies?

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SNPs associated with Charcot-Marie-Tooth disease (CMTX5) and Arts syndrome, clinical and heterogenous neurological disorders, have been mapped to the human *PRPS1* gene which encodes PRPP synthetase (Prs). Prs links carbon and nitrogen metabolism to produce PRPP (phospho-D-ribosyl-α-1pyrophosphate) required for the *de novo* and salvage pathways of purines and pyrimidines, tryptophan and histidine. CMTX5, a common inherited human disorder occurs with a frequency of 1 in 2,500. Arts syndrome affects mainly males at a frequency of 1 in 10^6 leading to death in early childhood. Symptoms of Arts syndrome are delayed motor development, ataxia and increased susceptibility to upper respiratory tract infections whereas CMTX5 is characterised by peripheral neuropathy. There are five paralogous *PRS* genes in yeast. *PRS1*, *PRS3* and *PRS5* may have arisen from the prototype PRS-encoding genes, *PRS2* and *PRS4*, by duplication followed by acquisition of insertions (NHRs). Genocopies which mimic the SNPs associated with CMTX5 and Arts syndrome were created to examine their impact on yeast physiology. Caffeine sensitivity and Rlm1 expression of the genocopies p.L115T (CMTX5) and p.Q133P (Arts syndrome) were increased at ambient temperature, indicating that these mutations may influence the folding of Prs1, thus impacting on the phosphorylation status of Slt2 and NHR1-1/Slt2 interaction, thereby linking primary metabolism with cell signalling. Deletion of *PRS1*, *PRS3* or *PRS5* causes sensitivity to lithium, a natural Gsk3 inhibitor, suggesting the involvement of Prs in neuropathology. NHR5-2 of Prs5 contains three neighbouring phosphosites which, when mutated, compromise Rlm1 and Fks2 expression and weakens the interaction of Prs5 with the kinase Rim11, one of the four yeast Gsk3 paralogues. Removal of NHR3-1 of Prs3 destabilises the Prs1/Prs3 heterodimer, leads to caffeine sensitivity, lowers Rlm1 expression and prevents rescue of a synthetically lethal *prs3Δ prs5Δ* strain. Since deletion of *PRS5* causes rapamycin resistance we postulate an interaction between TOR signalling and PRPP synthetase, thus adding a new dimension to the application of yeast research in the discovery of novel therapeutic targets for the treatment of the human neuropathies, CMTX5 and Arts syndrome.

89V

Testing all possible CPOX missense variants to proactively detect pathogenic variation

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Genome sequencing is a tool for diagnosing hereditary coporphyria (HCP), which can be caused by sequence variation in coproporphyrinogen oxidase (CPOX). However, sequence-based diagnosis is severely limited by the difficulty of distinguishing tolerated from damaging variants. Although more prevalent variants can be statistically associated with disease, most of the missense variants in CPOX (52.6% of those in ClinVar) have been interpreted as “variants of uncertain significance” (VUS). Here, we validate a yeast-based functional complementation assay for CPOX missense variation. Using saturation codon-mutagenesis, en masse growth selection, and sequencing, we applied the assay to all possible amino acid substitutions in CPOX, thus generating a comprehensive ‘variant effect map’. High-quality measurements of functional impact were obtained for 84% of all possible amino-acid substitutions, and for >90% of substitutions that are accessible by a single-nucleotide change. Importantly, we demonstrated the potential clinical utility of the CPOX variant effect map, showing that it can outperform all assessed computational methods in reliably identifying pathogenic missense variants. We show that yeast-based functional complementation assays for heavy metal tolerance can identify variants of CPOX associated with an atypical metal-induced porphyrinogenic response in humans. Thus, we are proceeding to generate an atlas of the impacts of CPOX variants across environmental contexts to enable more rapid and sensitive genetic diagnosis of HCP, support therapeutic intervention for an even larger number of individuals, and perhaps allow a gateway for 'genome-informed career counselling'.

90A

Natural variation in the consequences of gene overexpression during osmotic stress

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Free-living cells live in fluctuating environments and must be able to respond rapidly in order to survive. Genetic background can influence how an individual responds to a changing environment, including stressful changes. Changes in gene copy number can influence stress tolerance and responses, presumably due to corresponding changes in gene expression. However, we recently showed that strains vary in their ability to tolerate gene copy number differences; how this influences variation in stress tolerance is relatively unknown. Here we measured the tolerance to gene overexpression (OE) in four different *Saccharomyces cerevisiae* strains, including wild isolates, when strains were grown under osmotic and ionic stress induced by sodium chloride (NaCl). OE genes that were deleterious to most strains under NaCl stress were enriched for translation processes, similar to the enrichments of deleterious genes in rich, non-stress medium. We found that the West African NCYC3290 strain and the North American YPS128 strain are more sensitive to NaCl stress than other strains. These strains showed the greatest number of sensitivities to gene OE compared to other strains. Although most genes were deleterious in these strains, subsets of genes are highly beneficial when OE during NaCl stress. Many of these highly beneficial genes are functionally related and point to underlying differences in strain physiology. This work illustrates how tolerance to gene overexpression is influenced by genotype-environment interactions.

91A

Evolved lager laboratory hybrids exhibit high fermentation capacity under beer fermentation

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The interspecific hybrid *Saccharomyces pastorianus* (*S. cerevisiae* x *S. eubayanus*) is the main responsible for the lager beer fermentation. However, lager yeast strains have a low genetic diversity, affecting the repertoire of distinct lager beers. An alternative is the generation of novel strains for the diversification of lager beers, which could aid the understanding of the origin and domestication of *S. pastorianus*. Recent studies used a handful of strains to generate new lager hybrids, in particular a single *S. eubayanus* type strain, limiting the outcomes of these studies and impeding the deep understanding of the lager yeast domestication process. Alternatively, the recent isolation of hundreds of *S. eubayanus* and *S. cerevisiae* strains in Chile with high genetic and phenotypic diversity can overcome this obstacle through the selection of individual strains with specific desirable fermentation characteristics. Therefore, in this study we generated a large set of novel interspecific lager yeast hybrids using a genetically rich collection of wild Chilean strains for beer wort fermentation. For this, we selected three Chilean strains of *S. cerevisiae* and *S. eubayanus*, which were spored and mated at two temperatures (12 °C and 25 °C), generating 31 interspecific hybrids. The hybrids were phenotypically characterized under beer wort fermentation and growth under five fermentative conditions of interest. The hybrids showed similar fermentation capacities to their parental strains, with no evidence of positive heterosis. Still, new hybrids presented a wide diversity in growth rates when evaluated in different media, exhibiting efficient maltotriose consumption, greater than those observed in parental strains. To ameliorate the hybrid’s fermentative performance, four F1s were selected for improvement through a process of adaptive evolution under high ethanol concentrations. After 250 generations, the different evolved lines showed higher fitness in the same evolution environment than the ancestral hybrids. Although none of the evolved lines presented the same fermentation efficiency as the commercial strain, they considerably improved their fermentation rate compared to the ancestral hybrids, representing interesting lager hybrids for the beer industry. Consequently, this large set of new lager hybrids are a valuable source of genetic and phenotypic diversity for their utilization in breweries and/or used to understand the domestication process of lager beer.

92A

Population genomics of structural variations in *Saccharomyces uvarum*

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Ancient and wild forests represent great yeast biodiversity sources for the bioprospecting of wild yeast with novel phenotypes of industrial interest. *Nothofagus* forest stands as one of the most important wild areas in southern South America, representing a place with high levels of yeast diversity due to the biogeographic history of this region. *Saccharomyces uvarum* is a non-domesticated yeast widely distributed across the globe, particularly in South America. It recently received further attention for its fermentation profile in wine and cider, providing a distinct character to wines. The phylogenetic analysis of *S. uvarum* resolved three main clusters: (A) Holarctic, (B) South America, and (C) and Australasia. The Holarctic lineage originated from a restricted subset of a South America population and contains North American and European strains. The South American clade mainly contains strains from Patagonia, with the highest richness in terms of genetic diversity. The Australasia clade is from Oceania and shows the greatest genetic divergence, and partly reproductive isolated from the other lineages. Despite some preliminary information on structural rearrangements underlying genetic and phenotypic divergence between lineages, the overall extent of the structural variants in the lineage divergence in *S. uvarum* remains unclear. To this end, we surveyed 15 National Parks in Chilean Patagonia, ranging from the Andes Mountains up to the Pacific coast. Overall, we isolated over 500 different isolates and sequenced 50 of them using short and long-read technologies. Using orthologous sequences, we found that Chilean strains clustered into all the described lineages, except Holarctic. Interestingly, a subset of the strains isolated near the Pacific Coast clustered in the Australasia lineage, demonstrating that Patagonia contains the greater number of lineages in the species. In addition, we uncovered the genome-wide spectrum of structural variants segregating across lineages. In this way, we identified two significant translocations and one inversion in the Australasian clade, which could explain the partial reproductive isolation of the Australasia lineage. This genetic divergence translated into significant phenotypic differences, some of them with industrial potential. Our results support the native origin of this species to the Southern Hemisphere, highlighting the richness of Patagonia in terms of wild yeast´s genetic and phenotypic diversity.

93A

Exploring the role of RNA editing in overcoming short-term extreme environmental conditions

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Random genomic mutations combined with natural selection allow response to changing conditions and evolutionary adaptation of the genetic information to the varying needs dictated by the dynamic environment. However, fixation of a new allele, let alone a new trait, requires many generations. Thus, this powerful mechanism is ineffective for environmental changes happening on much shorter time scales.

RNA editing, a post-transcriptional process, modifies the RNA molecule while it carries the genetic information and passes it to the protein-producing ribosome. Thus, it alters the RNA sequence and allows proteome diversification beyond the genomic blueprint. Unlike genomic mutations, RNA edits are not directly transmitted to the next generation of cells. This feature may be extremely advantageous when the challenge is transient, and the required mutation rate is high. Overcoming these challenges at the DNA level requires a significant elevation of the mutation rate, leading, inevitably, to cell death. In contrast, transient RNA edits may provide a viable solution.

We therefore hypothesized that elevated RNA editing is utilized to increase transcriptomic diversity and bypass short-term evolutionary barriers. To demonstrate this idea, we created a selection-neutral in-vivo system to investigate the effects of massive RNA-editing on fitness and evolution. To this end, we engineered yeast cells to express the Adenosine deaminase acting on RNA (ADAR) RNA editing proteins that catalyze the deamination of adenosine nucleotides to inosines (A-to-I), which are then read as guanosines during translation. Yeast cell, whose origins precede the emergence of ADARs, lack the endogenous capacity of A-to-I editing but can express them. Remarkably, we identify extensive (thousands) A-to-I changes, transcriptome-wide, without leaving any traces on the DNA. The extensively edited cells were cultured for several hours under stresses that are known to impair cells' survival. The stress factor was then relieved, and the survival rate within the population was quantified by the number of colonies formed. The results confirmed that survival was obtained by editing a gene known to provide resistance to this stress.

These results highlight RNA editing as a novel key contributor to adaptation and acclimation, going beyond the classical genetic paradigm and providing a different angle to our understanding of the evolutionary process.

94A

Assessment of reproduction isolation and fitness variation across coexisting *Saccharomyces cerevisiae* pre-domesticated lineages

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Barriers to hybridization may be generated by the geographic distance or the intrinsic properties of the organism, such as hybrid incompatibility and outbreed depression. *Saccharomyces cerevisiae* is a good model for assessing reproduction isolation and measuring the fitness of strains and hybrids. In Fushan Botanical Garden in Taiwan, 23 isolates from 106 trees were collected within 200 meters in our previous study and are assigned to eight strains according to phylogenetic analysis. The eight strains belonged to four lineages and four mosaic genome structures with different ancestry compositions. Intriguingly, few hybrids from nearby lineages was found. Since these isolates coexist at a small spatial scale, the geographic distance may not play a major role in a barrier to hybridization. Hence, we aim to

establish a set of potential hybrids at this site and to delineate the hybridization barrier by assessing the degree of reproductive isolation and the fitness variation of the hybrids and their parents. For this, we first constructed haploid strains of eight isolates from different clonal groups by deleting the HO locus using the CRISPR-Cas9 system. The 100% efficiency of targeted mutation was confirmed by colony PCR. The stable haploids were then constructed from ∆ho tetrads and mating type was determined by mating with the reference strains ∆sst2 and ∆bar1. Next, the spore viability was calculated from dissected spores produced by pairwise crossing the haploids. The results showed that the spore viability ranges from 89 to 98% and varies between mating types. Finally, the fitness will be evaluated by measuring the growth rate in different culture conditions. In this study, the haploid resource of coexisted natural *S. cerevisiae* isolates provides us to identify their hybrid potential and hybrid fitness, elucidating the low-hybrid-event phenomenon in this limited area.

95A

Search for genes involved in adaptation to low nitrogen conditions for wine production in wild and domesticated strains of *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae is the main species responsible for alcoholic fermentation in the transformation of grape must into wine, one of the main problems being the deficiency of nitrogen sources in the must, which can lead to stuck or sluggish fermentations. An important challenge is to identify the genetic basis underlying the phenotypic variability in adaptation to low nitrogen conditions, especially considering the existing phenotypic and genotypic differences between wild and domesticated strains of *S. cerevisiae* studied to date. In the present work, we evaluated the adaptation of a wide yeast population to nitrogen-limited wine fermentation condition, exploiting the phenotypic diversity of the species using the most complete catalogue described so far, the population derived from "The 1002 Yeast Genomes Project", in which 1011 yeast strains from multiple ecological niches were isolated and their genomes fully sequenced. We then identified genes associated to nitrogen-limited wine fermentation by exploiting the genetic diversity of this population, confirming that it is a powerful resource for direct genotype-phenotype linkage using Genome Wide Association Studies (GWAS), which allowed us to associate polymorphisms and copy number variation (CNV) present in the population with the phenotypic information. The results obtained open up the possibility of improving an industrial wine yeast using the identified polymorphisms through cutting-edge genetic engineering techniques such as CRISPR-Cas9.

96A

The evolutionary divergence of transcription start sites (TSS) is shaped by the functional impact of 5' UTR (5' Untranslated Region) length

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The lengths of 5' UTR (5' Untranslated Region) are highly diverse among genes in a genome. Different 5'UTR lengths are commonly generated by the same gene due to alternative usage of transcription start sites (TSSs) in different tissues or responding to environmental stimuli. However, the mechanisms driving the diverse 5'UTR length and alternative 5'UTR usage remain controversial. Here, we sought to address this question by systematically analyzing the functional impacts of 5'UTR on gene expression using high-resolution quantitative TSS maps. In general, 5' UTR lengths negatively correlate with transcript abundance, and changes in 5'UTR length are also associated with gene differential expression. We also found that 5' UTR length negatively affects mRNA stability and translation efficiency. Furthermore, our evolutionary genomics study demonstrated that the evolution of sequences near TSSs had been shaped by natural selection. Based on these observations, we proposed a function model of 5'UTR length that provides a plausible explanation to the highly diverse 5'UTR length among genes and prevalent alternative 5'UTR length generated by a gene. In brief, new TSSs of a gene may be generated by stochastic mutations, generating transcripts with various 5'UTR lengths. Due to its functional impact on the gene expression outcomes, 5'UTR length variants result in different fitness effects. Specifically, shorter 5'UTRs are beneficial for genes with higher protein demand or vice versa. Thus, the fixation of a 5'UTR length variant is largely determined by its fitness consequences, improving our understanding of gene regulatory mechanisms and evolution of gene structure.

97A

The effect of cis-acting elements on copy number variant formation and dynamics during adaptive evolution

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Detecting and predicting heritable changes in DNA that lead to adaptation is an essential goal in evolutionary biology. Copy number variants (CNVs) -- gains and losses of genomic sequences -- are a pervasive class of mutation and source of genetic variation that frequently underlie rapid adaptation. Although mechanisms of CNV formation have been identified, the role of local genomic architecture on CNV formation, selection, and subsequent dynamics remains elusive. To begin answering this question, we investigated the effect of proximate elements of general amino acid permease gene, *GAP1*, on CNV formation and dynamics in *Saccharomyces cerevisiae*. *GAP1* is flanked by two long terminal repeat (LTR) elements and has one downstream autonomously replicating sequence (ARS) element. First, we engineered strains lacking either an ARS, two LTRs, or all three elements in the background of an existing *GAP1* CNV reporter strain, wherein a constitutively expressed fluorescent mCitrine gene was inserted adjacent to *GAP1* (Lauer et al 2018). Then we experimentally evolved these strains in glutamine-limited chemostats for 200 generations and detected *GAP1* CNVs using this single-cell resolution CNV reporter system.

Our study validated that these elements were indeed cis-acting to *GAP1* CNV formation. Our results recapitulate previous findings that *GAP1* CNVs are repeatedly generated and selected for early during adaptive evolution before undergoing more complex dynamics. We found the engineered mutants have different dynamics relative to the wild type, surprisingly appearing to reach higher copy numbers. This suggests mutants lacking any of these cis-acting elements employ different mechanism(s) to generate higher copy CNVs. Whole-genome-sequencing of isolated clones by a mixture of Nanopore and Illumina methods revealed CNV structure. Subsequent breakpoint analysis uncovered the mechanisms prevalently used. This study brings us closer to understanding the role of local genomic architecture on the evolutionary dynamics of adaptation.

98A

Homology and Disease curation at SGD: budding yeast as a model for eukaryotic biology Stacia R Engel, Robert S Nash, Marek S Skrzypek, Edith D Wong, Suzi Aleksander, Shuai Weng, Kalpana Karra, Stuart Miyasato, J. Michael Cherry Stanford University

The foundation for much of our understanding of basic cellular biology has been learned from the budding yeast *Saccharomyces cerevisiae*. Studies with yeast have also provided powerful insights into human genetic diseases and the cellular pathways in which they are involved. Here we present an update on developments at the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/), the premier community resource for budding yeast. SGD includes high quality manually curated information regarding human orthologs and associated diseases, homologs in other model organisms, and functional complementation between yeast and human genes. This information is provided in meaningful ways allowing data mining and discovery by integrating these data into this encyclopedic online resource. We also highlight other popular data, such as written summaries about yeast genes and their mutant phenotypes, their human homolog disease associations, and presentation of the yeast/human/model organism ortholog set. SGD maintains these different datatypes, and distributes them to the scientific community via the web and file transfer. These curation efforts are part of our continuing mission to educate students, enable bench researchers, and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

99B

Reversion dynamics of copy number variants in the absence of selection Titir De, David Gresham Biology, New York University

Copy number variants(CNVs)–duplications and deletions of genomic sequence– are a major source of natural genetic variation among individuals. Due to their large phenotypic effects, CNVs drive rapid adaptive evolution under strong selection pressures. CNVs provide fitness benefits by altering gene expression, causing outcomes like increased nutrient transport in microbes and increased disease defence in plants. However, CNVs can also be deleterious under conditions where they are not selected.

We use general amino acid permease gene (*GAP1*) as a model CNV-associated gene in *Saccharomyces cerevisiae*. Prior research shows that adaptive CNVs arise rapidly under certain selective pressures. Evolution in glutamine limitation repeatedly selects for *GAP1* CNVs, since increased *GAP1* copies improve fitness of those strains under nutrient limitation. However, it is unknown whether acquired CNVs are maintained or lost when that pressure is removed. We tested the hypothesis that in conditions without positive selection, fitness costs of increased gene copy numbers outweigh their survival benefits, so CNVs are lost, causing reversion to the ancestral single copy number.

First, we tested the fitness of 5 different CNV-containing yeast strains in rich media with no nutrient limitation, using 3 independent replicate populations per strain. All 5 strains had evolved from the same ancestor containing 1 *GAP1* copy, and they all had 2 or more *GAP1* copies, but CNV structures differed between strains. In the absence of selection, we find that these strains have lower fitness than the single-copy ancestor, as reflected in reduced growth rates. We evolved the strains in rich media for ~150 generations, tracking *GAP1* copy number via a fluorescent reporter gene. 8 out of 12 populations show rapid CNV loss, usually after 50 generations. Of these 8 populations, 4 revert to a single *GAP1* copy, but others show complete deletion of all *GAP1* copies.

Since *GAP1* is expressed only in nitrogen limitation and is repressed in rich media, our results suggest that selection against *GAP1* CNVs in rich media is not due to *GAP1* expression costs. We are using whole genome sequencing and mutagenesis to test whether negative selection is due to costs of other genes within the CNV or due to amplification-associated genome instability. Our results indicate that in the absence of positive selection, it is frequently beneficial for cells to lose extra gene copies that were acquired under strong selection, highlighting the transient and dynamic nature of copy number variation.

100B

Evolutionary constraints override mutational constraints in redomestication of wild *S. cerevisiae* and *S. paradoxus* Emery R Longan, Justin C Fay Biology, University of Rochester

Evolutionary constraints limit adaptation by natural selection. Among closely related species, constraints are often quite consistent regarding the capacity to adapt to novel stressors such as insecticides or antibiotics. However, species' constraints change as they diverge, which can lead to long term evolutionary consequences such as historical contingency. Despite the presence of differential constraints between species, the cause of these differences is often elusive. Mutational constraints, which refer to limits placed on adaptation due to certain phenotypes being mutationally inaccessible, are often confounded with other types of evolutionary constraints such as pleiotropy and epistasis. *S. cerevisiae*, unlike its sister species, *S. paradoxus,* has adapted to the oenological stressors copper and sulfite. Both species are commonly isolated from vineyards and presumably have both been subject to selection for resistance to both antimicrobials. To test for and to distinguish between mutational and other evolutionary constraints in this system, we performed two sets of experiments. First, we performed genome wide saturation mutagenesis in both species and recovered mutants resistant to copper and sulfite. Second, we

performed experimental evolution of 720 populations of both species on media containing these stressors for 70 passages. Resistant strains derived from both studies were phenotyped for copper and sulfite resistance, and a subset were subjected to whole genome sequencing. We found a stark contrast in the results derived from these two approaches. Mutagenesis showed a small but significantly greater mutational effect size of copper mutants in *S. paradoxus*, whereas experimental evolution resulted in greater resistance of *S. cerevisiae* in both stressors. Genome sequencing showed that although genetic parallelism is common between the two species at both the gene and site level, there are many subtle differences in the distribution of mutational effects, including varying effect sizes of mutations within the same genes, and different genes being the targets of selection. Yet, these mutational differences were not sufficient to predict evolutionary outcomes, which depend not only on the first step of adaptation, but also subsequent steps, epistasis, and competition among lineages. Our study demonstrates that other evolutionary constraints can override mutational constraints and play a dominant role in evolutionary outcomes.

101B

Long-term adaptation to a secondary carbon source in *Saccharomyces cerevisiae*

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The principle of catabolite repression is a core concept of metabolic gene regulation in microorganisms. When glucose is present, genes encoding enzymes required to utilize alternative carbon sources, such as galactose, are subjected to catabolite repression. Galactose is a secondary fermentable sugar that requires three regulatory genes and four structural genes for its assimilation. In the presence of only galactose, the catabolic repression is attenuated, and the structural *GAL* genes are fully activated. We asked how cells respond to long-term propagation in galactose as a sole carbon source.

Here, we performed a 4,000-generation evolution experiment using 48 diploid *Saccharomyces cerevisiae* populations to study adaptation in galactose. We show that fitness gains were greater in the galactose evolved population than in identically evolved populations with glucose as a sole carbon source. Whole-genome sequencing of 96 evolved clones revealed recurrent *de novo* single nucleotide mutations in candidate targets of selection, copy number variations, and ploidy changes.

We find that most mutations that improve fitness in galactose lie outside of the canonical *GAL* pathway and are involved in nutrient signaling. Reconstruction of specific evolved alleles in candidate targets of selection, *SEC23* and *IRA1*, showed a significant increase in fitness in galactose compared to glucose. In addition, most of our evolved populations (28/46; 61%) fixed aneuploidies on Chromosome VIII, suggesting a parallel adaptive amplification. Finally, we show greater loss of extrachromosomal elements in our galactose-evolved lineages compared with previous glucose evolution. Broadly, these data further our understanding of the evolutionary pressures that drive adaptation to less-preferred carbon sources.

102B

Experimental evolution for investigating the genetic basis of freeze-thaw tolerance

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A major challenge encountered across the many applications of *S. cerevisiae* is how to store yeast samples for long periods of time while maintaining maximal viability. In molecular genetics research, it is common practice to freeze cells; however, freezing samples can be damaging, resulting in low cell survival upon thawing. Although freezing is a ubiquitous practice, there remains sparse research on freeze-thaw stress in yeast; a comprehensive list of freeze-thaw response genes and an understanding of how variation at these loci affect freeze-thaw phenotypes is still lacking. **The main goal of this project is to elucidate genetic factors that play a role in yeast freeze-thaw tolerance and functionally characterize variation in those factors across the species.** I will identify causative loci by selection of *de novo* mutations from a freeze-thaw evolution experiment and investigate fitness differences caused by natural variation at genes involved in the freeze-thaw response.

The freeze-thaw experimental evolution approach involves subjecting a clonal population of yeast to repeated cycles of freezing, thawing, and growth. After several cycles, random mutations that confer increased freeze-thaw tolerance will be selected for, and the population is expected to attain increased freeze-thaw viability. Whole genome sequencing analysis of the ancestor and evolved samples will determine the responsible genetic changes for improved freeze-thaw tolerance.

Once genetic factors for freeze-thaw tolerance are identified through experimental evolution, the 1,011 *S. cerevisiae* strain collection will be utilized to assay species-wide variation at these loci for both sequence and function. Our lab has developed a high-throughput method for functionalizing natural variants of a gene by cloning barcoded alleles en masse into the lab strain, then competing the transformants under a condition of interest. This approach will be used to characterize allelic variation in freeze-thaw tolerance genes, starting with *AQY1* and *AQY2*, which are already known to be important for this trait.

This work will give us a comprehensive list of factors involved in freeze-thaw tolerance and will provide a better understanding of the specific effects of variation at freeze-thaw tolerance genes. Furthermore, generating an experimentally evolved strain with improved freeze-thaw tolerance compared to existing strains will have many important applications in both research and many other industries.

103B

Characterization of *Candida albicans* error-prone polymerases in antifungal drug resistance and DNA damage

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Candida albicans is a commensal yeast that exists naturally on human skin and mucosal surfaces. Despite its ability to exist harmlessly in human hosts, it is considered an opportunistic pathogen and can cause severe and life-threatening disease in immunocompromised individuals. It is the fourth most common cause for nosocomial bloodstream infections and is the leading cause of deaths associated with invasive fungal infections. *C. albicans* is a successful pathogen due to a number of virulence mechanisms, and the lack of effective antifungal therapies coupled with the rising incidence of antifungal drug resistance has established this organism as a significant threat to human health. Advancements in gene editing technologies, such as CRISPR, have provided efficient means by which *C. albicans* can be studied to help identify novel antifungal drug targets and explore possible avenues for slowing antifungal drug resistance. In bacteria, there is a well-established phenomenon linking treatment with antibiotics to increased rates of mutagenesis and drug resistance, mediated by error-prone polymerases. These polymerases are upregulated upon stress-induced DNA damage. They facilitate rapid DNA repair and confer tolerance to DNA damage while introducing mutations into the genome, ultimately driving drug resistance. Although error-prone polymerases have been identified and researched in the model yeast organism *Saccharomyces cerevisiae*, they have yet to be characterized in pathogenic yeast such as *C. albicans*. We have generated deletion mutants of genes encoding error-prone polymerases in *C. albicans* based on known orthologs in *S. cerevisiae* and profiled their ability to influence mutation rate and evolution of antifungal drug resistance. This study aims to determine the role of error-prone polymerases in the DNA damage response pathway, and in mediating mutagenesis-based antifungal drug resistance.

104B

Exploring the evolutionary arms race between human PKR and vaccinia K3L

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The interface between interacting host and viral proteins can be a battleground in which genetic variants are naturally pursued. One such scenario is found between the mammalian innate immunity protein PKR (protein kinase R) and its poxvirus antagonist K3. Exploring the impact of missense variants in both PKR and K3 will highlight residues of evolutionary constraint and opportunity while also elucidating the mechanism by which human PKR is able to subvert a rapidly evolving antagonist. We reason that paired human *PKR* and vaccinia *K3L* variants can be characterized using a combinatorial high-throughput cloning approach and a yeast growth assay. In this assay, PKR activity suppresses yeast growth, which is restored if K3 successfully inhibits PKR. By tracking barcodes from sample timepoints in the assay we will be able to quantify and characterize the impact of each *PKR* and *K3L* variant combination, highlighting points of evolutionary constraint an opportunity for *PKR* and *K3L*. This strategy would allow us to scan a vast combinatorial space in a single experiment, providing details of the evolutionary fitness landscape of *PKR* and *K3L* as well as the ability of each protein to adapt to the other.

105B

Expression of heterologous ADAR enzymes in yeast indicates editing activity is affected by temperature, and identifies the mdADAR1 as a hyper transcriptome diversifier

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The most abundant form of RNA editing in metazoans is the deamination of adenosine to inosine (A-to-I), which is mediated by the Adenosine Deaminase Acting on RNA (ADAR) enzymes, that binds double-stranded RNA (dsRNA) structures. ADAR mediated A-to-I editing which allows genomically-encoded nucleobases to be transformed and differently recognized in the RNA sequence is a powerful means of creating inner transcriptome diversity and this makes it an attractive therapeutic tool.

One of the main challenges in the field are attempts to increase editing efficiency on difficult targets. Since ADARs are expressed in all metazoans, we speculated that unique features that evolved based on adaptation, driven by the ecosystem they inhabit could be identified and exploited. Since genomes that express the ADAR proteins were subjected to selective pressures, it is challenging to differentiate its intrinsic substrate preference. To address this issue, we used as a neutral testing ground the yeast *Saccharomyces cerevisiae*, an organism whose origin precedes the emergence ADARs and therefore doesn't possess the endogenous capacity of A-to-I editing.

The exogenous expression of heterologous ADARs in yeast resulted in extensive A-to-I changes, implying that they carry out their biological function. We also detected a clear association between growth impairment and transcriptome editing levels implying that it can be used as a screening platform, to reveal their inherent and distinct editing potential. Notably, we found that the expression of a mallard duck and hummingbird ADARs originated from birds with a core temperature of 42ºC, the warm end of the endothermic spectrum, exhibit exceptionally high A-to-I activity. Since most sites have decreased A-to-I editing levels at higher temperatures, primary as a result of RNA structure destabilization, we suggest that one factor that may contribute to higher temperature adaptation is ADAR adaptation resulting in versions with greater catalytic activity, or that better recognize their dsRNA substrates. In most cases, although there are potentially many editing sites, this massive editing tool is of little use in the natural cellular environment and can be uncovered by using yeast cells as a neutral system. We envision that our screening platform should form the basis for identifying the factors that govern ADAR activity and consequently to engineer the human protein making the final product customizable to meet specific requirements.

106B

Effects of domestication on the sexual reproduction of *Saccharomyces Cerevisiae*

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Brewing yeast is primarily asexually reproducing. When yeast are in nutrient depleted conditions, they sporulate to produce haploid offspring. Domestication has caused brewing yeast to be selected for favorable traits. The ability to produce asexually allows for a more efficient brewing process. Within the group of brewing yeast, there are multiple clades that are distinguished by distinct markers of domestication. Our project aims to investigate the ability of brewing yeast to sporulate and if those offspring are then viable to reproduce. We will test a variety of sporulation methods to test the efficiency and viability of the brewing strains. In the future, we hope to be able to investigate the connections between brewing phenotypes and the ability to use sporulation as a stress response. We hope to gain a better understanding of the relationship of domestication events and the adaptations that strains have acquired.

107V

Evolution of the Pleiotropic Drug Resistance (Pdr) Snq2/Pdr18 gene subfamily in the hemiascomycete yeasts

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The transporters of the ATP-Binding Cassete Pleiotropic Drug Resistance (ABC-PDR) family are important genetic determinants of Multidrug Resistance (MDR) in the Hemiascomycetes. These proteins divide into ten phylogenetic clades, one of which comprises the members of the Snq2/Pdr18 subfamily. The *PDR18* gene encodes a transporter involved in the control of the ergosterol content present in the plasma membrane, conferring MDR to ethanol, acetic acid, pesticides and metal cations [1]. The *SNQ2* gene that also confers MDR but to different sets of chemical compounds with little overlapping [1]. A past study showed that a duplication event occurring in the common ancestor of the *Saccharomyces* genus was at the origin of the *PDR18* and *SNQ2* genes [1]. In the following of this study, the members of the Snq2/Pdr18 subfamily were identified in 171 yeast strains (68 species), with 263 full-size proteins being identified in these genome sequences. All early-divergent yeast species analyzed in this work lack Snq2/Pdr18 homologs, suggesting that the origin of this type of ABC-PDR genes in the hemiascomycete yeasts was a horizontal transfer event. A Comparative Genomics methodology allowed the reconstruction of the evolutionary history of the Snq2/Pdr18 homologs encoded in the pathogenic *Candida* species, showing the existence of a single main gene lineage leading to the *C. albicans SNQ2* gene. An entropy-based approach combined with moving average functions allowed detecting all the cytoplasmic nucleotide-binding domains known to be present in the ABC-PDR proteins and six new protein motifs that seem specific to the Snq2/Pdr18 homologs. Three models of molecular evolution were used to analyze the past action of selective forces over the members of this subfamily. The aBSREL model and a new approach combining two algorithms, the MEME model and MrBayes ability of reconstructing ancestral sequences, showed that the first *PDR18* ortholog have been under strong diversifying selection. The gathered results also suggested that a small portion of the sequence of the first *SNQ2* ortholog might have been under mild positive selection. Strong positive selection was exerted over one of the two paralogs generated by the Whole Genome Duplication (WGD) event, corresponding to the duplicate at the origin of a “short-live” WGD sub-lineage (spanning only Tetrapisispora and Vanderwaltozyma species). The other post-WGD duplicate did not showed signs of the past action of positive selection suggesting that the physiological function of the pre-duplication gene was preserved in the genes comprised in the “long-live” WGD sub-lineage. This study allowed the identification of amino acid residues with evolutionary importance in these ABC-PDR transporters, potentially relevant for the physiological function that the Snq2/Pdr18 homologs play in yeast cells.

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108V

*MAT*α-composing genes for sex-determining transcription factors evolved as haploinsufficient genes monitoring genome size in *Saccharomyces cerevisiae*

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Sexual dimorphism controlled by the sex-determination system is ubiquitous among eukaryotic organisms from yeasts to humans. Genetic analysis has allowed extensive analysis of the regulation of dichotomous sex and reproduction in the budding yeast *Saccharomyces cerevisiae*. *S. cerevisiae* haploid cells show either an a- or α- mating type; mating two cells of different mating types produces an a/α diploid. Yeast mating types are determined by the mating type (*MAT*) locus, which is located on chromosome III and contains either the *MAT*a or *MAT*α idiotype. However, *MAT*a is not involved in the determination of the haploid mating type. Mating type of haploid cells is determined by the presence or absence of the *MAT*α containing *MATα1* and *MATα2* genes, which encode the transcription factors responsible for sex-specific gene expression. These transcription factors are characterized by rapid turnover, which is thought to be important for the progression of the mating type switching during the homothallic life cycle. However, it is not clear how sex-determining genes contribute to sexual stability. In this study, we devised a method of controlling the levels of intracellular sex-determining transcription factors using polyploid yeast cells and addressed the relationship between their intracellular levels and sexual stability. Polyploid cells with fewer *MAT*α copies had unstable sexual phenotypes, causing morphological changes and an increase in cell death; these effects were mediated by hyperactivation of the mating pheromone response signaling pathway. This indicates that the *MATα1* and *MATα2* genes are haploinsufficient genes requiring strict quantitative control, and that it is important for stable expression of the sexual phenotype. Because chromosome III is equivalent to sex chromosomes in other eukaryotes, the *MAT* locus and chromosome III may be involved in speciation, analogous to sex chromosomes in higher eukaryotes. Chromosome III is among the shortest of 16 chromosomes in *S. cerevisiae*, and the

most prone to loss in diploid yeast. We propose that strict quantitative control of the *MAT*α products in *S. cerevisiae* compensates for the drop-out prone nature of chromosome III, thereby suppressing speciation through increased genome size via polyploidization.

109V

Gene whose overexpression is adaptive illuminates the missing pieces of the cell

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Genome-wide overexpression studies have revealed the mechanism of negative cellular effects of perturbations to expression levels (Sopko 2006, Makanae 2013). In contrast, overexpression of genes also can enhance cellular fitness under stress, and has been well studied for the purpose of identifying drug targets (Rine 1991, Ho 2009). In this study, we considered what nature of the cells are revealed by studying overexpression-driven adaptations to environmental stresses, which are considered to have more complex effects than drugs.

To identify genes whose overexpression is functionally adaptive (GOFAs) in about 5,700 genes of S. cerevisiae under various environments, we developed a high-throughput method designated ADOPT, using competitive culture and nanopore sequencing of a genome-wide overexpression collection. The identification of GOFAs in several environments showed that GOFAs varied not only with large differences but also with small differences in genetic backgrounds and environments. For instance, the small differences could be derivatives of the same strain, or with or without small amounts of additives under the same stress environment. This indicates that GOFAs strongly reflect genetic backgrounds and environments.

In this study, we specifically focus on GOFAs under NaCl stress. We utilized two laboratory strains, BY4741 and CEN.PK2-1C, with different salt tolerance and different genetic backgrounds. We found that GOFAs under 1 M NaCl were different between two strains and had functions related to calcium and potassium homeostasis; ECM27 and GDT1 in BY4741, and SAT4, HAL5, and ECM27 in CEN.PK2-1C, respectively. The addition of calcium in BY4741, and the addition of calcium and potassium in CEN.PK2-1C increased fitness under NaCl stress. This strongly suggests that GOFAs have the functions of compensating for deficiencies in the cell, in this case calcium and potassium. We argue that a genome-wide identification of GOFAs reveals what is deficient for the cell under the interaction of genetic backgrounds and environments.

110V

The neutral rate of whole-genome duplication varies among yeast species and their hybrids

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Hybridization and polyploidization are powerful mechanisms of speciation. Hybrid speciation often coincides with whole-genome duplication (WGD) in eukaryotes. This suggests that WGD may allow hybrids to thrive by increasing fitness, restoring fertility and/or increasing access to adaptive mutations. Alternatively, it has been suggested that hybridization itself may trigger WGD. Testing these models requires quantifying the rate of WGD in hybrids without the confounding effect of natural selection. Here we show, by measuring the spontaneous rate of WGD of more than 1300 yeast crosses evolved under relaxed selection, that some genotypes or combinations of genotypes are more prone to WGD, including some hybrids between closely related species. We also find that higher WGD rate correlates with higher genomic instability and that WGD increases fertility and genetic variability. These results provide evidence that hybridization itself can promote WGD, which in turn facilitates the evolution of hybrids.

111A

WOR1, the master transcriptional factor in the positive transcriptional feedback loop, is required for the maintenance of opaque cell type in an epigenetic manner

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Cell types can be epigenetically maintained for many generations, and the molecular mechanism underlying it is still under rigorously studied. Transcriptional feedback loop has been proposed to be able to establish and maintain cell types in an epigenetic manner. White cell and opaque cell, the two inheritable cell types in *Candida albicans* that differ in cellular morphology, mating efficiency, transcriptional profile as well as metabolism, has been a great model to study the mechanism underlying the maintenance of cell type. Although stable, the two cell types are reversible. Ectopic expression of WOR1, the master transcriptional factor in opaque cell, can induce the white-to-opaque cell type switch and establish the opaque transcriptional feedback loop. However, whether WOR1 and opaque transcriptional feedback loop are required for the maintenance of opaque cell type is unclear. Applying auxin-inducible degron system to WOR1 in opaque cell, we real-time induced and observed the decrease in WOR1 protein level, followed by the gradual transition of cellular morphology and transcriptional profile from opaque cell to white cell. This work serves as a direct evidence to proof that WOR1 protein as well as the transcriptional feedback loop are required for the maintenance of opaque cell type in *Candida albicans*. Furthermore, we also showed that the threshold of WOR1 protein level does exist to buffer the fluctuation of WOR1 protein level in order to maintain opaque cell type.

112A

Using SPRITE to investigate heat shock-dependent changes in 3D chromatin architecture and genome topology in *Saccharomyces cerevisiae*

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Chromatin architecture critically impacts nuclear processes that occur in a precise spatio-temporal manner such as gene transcription, replication, DNA repair and genetic recombination. Perturbations in chromosomal topology can lead to human disease, including developmental deformities and cancer. The response of budding yeast to acute thermal stress ("heat shock") is a dynamic example of transcriptional control and 3D genome organization. The evolutionarily conserved DNA-binding protein, Heat Shock Factor 1 (Hsf1), is a sequence-specific activator that orchestrates the heat shock response in eukaryotes from yeast to human. Previous studies in our lab using chromosome conformation capture (3C) and single-cell imaging showed that in response to acute thermal stress, Hsf1-dependent *Heat Shock Protein* (*HSP*) genes engage in robust intergenic interactions across and between chromosomes, forming coalesced foci inside the nucleoplasm. Such restructuring is dependent on Hsf1 and RNA polymerase II and is correlated with transcriptional activation. Moreover, these interactions are specific to heat shock-induced *HSP* genes as neighboring genes and genes activated by alternative transcription factors fail to coalesce. Recent work suggests that the coalesced *HSP* gene foci are part of dynamic biomolecular condensates that undergo liquid-liquid phase separation in response to heat shock.

To better understand the long-range intra- and interchromosomal interactions that take place during thermal stress, we are establishing the non-proximity ligation-based technique, Split Pool Recognition of Interactions by Tag Extension (SPRITE), in budding yeast. SPRITE has been described by M. Guttman and co-workers (Quinodoz et al, *Nature Protocols* 17:36-75 (2022)) for cultured mammalian cells and has been used to identify chromosomal interactions that occur within higher-order (beyond pairwise) structures within the mammalian nucleus. Using the SPRITE technique, we will test whether previously identified heat-shock and Hsf1-dependent intra- and interchromosomal interactions in yeast can be recapitulated using SPRITE. Moreover, we will explore the role of the Pol II CTD (including its phosphorylation state), chromatin remodeling factors and architectural proteins, and potentially other factors, in driving the dynamic genome-wide restructuring that our preliminary 3C analysis suggests. We are currently investigating the efficacy of using long-arm cross-linkers (e.g., DSG and EGS with arm lengths of 7.7 Å and 16.7 Å, respectively) in combination with formaldehyde (2 Å arm length) to obtain optimal preservation of 3D chromatin structure during the course of heat shock.

Keywords: Chromatin architecture, transcription, Hsf1, SPRITE, inter-chromosomal interactions.

113A

Identify proteins that bind to a specific mRNA in the cell cycle using dCas13d-APEX2 fusions

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Profiling the repertoire of proteins associated with a given mRNA during the cell cycle is unstudied. Furthermore, even from asynchronous cells, it is much easier to ask and answer what mRNAs a specific protein might bind to than the other way around. Recently, however, CRISPR-Cas and other technologies have been developed for proximity-labeling approaches engineered to target specific mRNAs 1. For the first time in any system, we implemented this technology at different points in the cell cycle in highly synchronous yeast cultures. We had previously shown that the translational efficiency of the FAS1 mRNA, encoding fatty acid synthase, peaks late in the cell cycle 2, and translational upregulation of FAS1 accelerates nuclear division 3. To understand how the translation of FAS1 is cell cycle-regulated, we identified proteins that bind the FAS1 transcript in a cell cycle-dependent manner. We used dCas13d-APEX2 fusions to target FAS1 and then label nearby proteins, which were then identified by mass spectrometry. We will discuss our findings from this novel, mRNA-centric technology for studies of mRNA-protein interactions.

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5.

114A

Natural variation in codon bias and mRNA folding strength interact synergistically to modify protein synthesis rates in *Saccharomyces cerevisiae*

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Codon bias and mRNA folding strength (mF) are hypothesized molecular mechanisms by which polymorphisms in genes modify protein synthesis rates. Natural patterns of codon bias and mF across genes as well as effects of altering codon bias and mF suggest the influence of these two mechanisms may vary depending on the specific location of polymorphisms within a transcript. Despite the central role codon bias and mF may play in natural trait variation within populations, systematic studies of how polymorphic codon bias and mF relate to protein synthesis rate variation are lacking. To address this need, we analyzed genomic, transcriptomic, and proteomic data for 22 *Saccharomyces cerevisiae* isolates, estimated protein synthesis rates for each allele of 1620 genes as the log of protein molecules per RNA molecule (logPPR), and built linear mixed effects models associating allelic variation in codon bias and mF with allelic variation in logPPR. We found codon bias and mF interact synergistically in a

positive association with logPPR through unknown mechanisms. We found codon bias has no independent effects while mF has negative independent effects on logPPR, consistent with decreased elongation rates due to longer ribosome pausing for more stable RNA secondary structures. We examined how the locations of polymorphisms within transcripts influence their effects and found that codon bias primarily acts through polymorphisms in domain encoding and 3' coding sequences, consistent with enhancing effects of slow translating codons in 5' and inter-domain sequences. mF acts most significantly through coding sequences with antagonistic interactions between coding sequences and UTRs. Our results present the most comprehensive characterization to date of how polymorphisms in transcripts influence protein synthesis rates.

115A

Transcriptome fates in response to starvation are regulated by splicing

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All organisms have evolved robust mechanisms for responding to changing cellular environments. Response to nutrient deprivation is coordinated by rapidly changing transcription and translation to quickly alter metabolic pathways to better equip the cells for changing nutrient conditions, and yet our understanding of how the molecular mechanisms that span across multiple processes remain obscure. Glyphosate, the active ingredient in the herbicide Roundup (GBH), inhibits aromatic amino synthesis, creating a starvation signal similar but distinct to exposure to rapamycin. From quantitative trait loci analysis in yeast differentially sensitive to GBH (both a lab and agricultural isolates), we identified variants in the *MUD1* gene, which encodes a nonessential splicing factor in the U1 snRNP. Loss of no other non-essential U1 protein confers resistance to amino acid starvation while loss of TRAMP complex increases sensitivity to starvation. The TRAMP complex degrades polyA transcripts in the nucleus and starvation sensitivity is repressed by also knocking out Mud1. The most downregulated transcripts in starvation encode ribosomal protein genes (RPGs) and RPGs contain most of the introns in the *S. cerevisiae* genome. The spliceosome is limiting in the cell and one of the ways non-essential energy-intensive processes are regulated during starvation is by inefficient splicing. The splicing index for the transcriptome was calculated when yeast were starved with glyphosate, depleting nutrients in the cell. The U3 snoRNA is an intron-containing Box C/D snoRNA required for processing the pre-rRNA into the 18S rRNA of the small ribosomal subunit. There are two paralogs that are expressed at different levels and have many polymorphisms in the intron. Splicing of the minor allele (U3b) is reduced to a greater extent during starvation. The minor allele has numerous SNPs and it is unknown if U3b functions differently than the higher expressed major allele. Regulation of splicing during starvation allows cells to rapidly reallocate resources away from energy-intensive ribosome biogenesis when resources are limited.

116A

Docosahexaenoic acid modulates intracellular *myo*-inositol and its biosynthetic genes.

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Bipolar disorder is a debilitating mood disorder characterized by recurring episodes of mania and depression. It affects 2.6% of adults and has a lifetime prevalence among adults of 3.9%. Current mood stabilizers are not always effective and/or are not well tolerated by many patients; thus, there is a need to develop or identify more effective and less harmful treatments. Omega-3- fatty acids have been shown to be effective in the treatment of bipolar disorder; however, their mechanism of action is unknown. *Myo*-inositol depletion has been hypothesized as the mechanism by which mood stabilizers exert their therapeutic effect. Using an enzymatic assay, we determined intracellular *myo*-inositol levels increased more than 2-fold when cells were grown in the presence of the omega-3 fatty acid docosahexaenoic acid (DHA). RT-qPCR was used to characterize the effects of DHA on genes in the *myo*-inositol biosynthetic pathway. We show DHA increases relative expression and has a concentration-dependent impact on *INO1* and *INM1*, which encode *myo*-inositol-1-phosphate synthase and *myo*-inositol monophosphate 1-phosphatase, respectively. We therefore conclude that the omega-3- fatty acid DHA exerts its therapeutic effect on bipolar disorder by increasing intracellular *myo*-inositol, which may be accomplished by upregulating its biosynthetic genes.

117A

Study of the cellular component-dependent functions of Xrn1

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The main decay pathway of yeast mRNAs in the cytoplasm uses the 5'→3' exonuclease Xrn1 (1). This protein shuttles from the cytoplasm to the nucleus, where it has a role as transcription factor (2). We have recently demonstrated that nuclear importing depends on two nuclear localization sequences (NLS1 & NLS2) and that exporting depends on its binding (presumably co-transcriptional) to mRNAs (3). It is also known that Xrn1 is able to degrade de-capped mRNAs that are still being translated by ribosomes (4). In this work, we analyze the pleiotropic functions of Xrn1 by comparing the phenotypes of yeast strains lacking Xrn1 or its capacity to be imported into the nucleus with those of its substitution by a cytoplasmic version of the paralogous 5'→3'exonuclease Rat1 (cRat1). We find that most of the global phenotypes of an *xrn1* mutant are partially complemented by cRat1, indicating that this 5'→3'-exonuclease has a similar enzymatic capacity as Xrn1, and that the lack of a cytoplasmic 5'→3'-exoribonuclease is the cause of the physiological defects of an *xrn1* mutant. The capacity of cRat1 to perform co-translational decay is, however, very limited. The comparison with the strain carrying Xrn1 without NLSs ($Xrn1^{\Delta NLS1/2}$) shows that it is slightly deficient in 5'→3'-co-translational decay compared to WT, but much more efficient than cRat1. These results indicate that Xrn1 shuttling, which is absent in both cRat1 and $Xrn1^{\Delta NLS1/2}$, is necessary for the full *in vivo* function of a cytoplasmic 5→3 exonuclease. We also hypothesize that the compromised co-translational decay abilities of cRat1 might be related to it hindering the shuttling of other decay factors.

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118A

Cryptic translation events target yeast ncRNA transcripts for nonsense-mediated decay

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The nonsense-mediated mRNA decay (NMD) pathway targets mRNAs undergoing premature translation termination for degradation. The *upframeshift* (*UPF*)*1-3* genes are required for NMD and their loss causes stabilization of targeted mRNAs. Surprisingly, many genes that are targeted by NMD (88%) appear to lack premature stop codons. One explanation for this targeting is that translation of cryptic ORFs results in widespread premature termination events. To test this hypothesis, we developed a combined approach using RNA-seq, to identify alternative transcript isoforms that are targeted by NMD, and ribosome profiling to find cryptic translation events. In particular, we used 40S footprint peaks on start and stop codons to demarcate the boundaries of ORFs that lead to NMD with high sensitivity.

Using this approach, we identified novel translation events that likely trigger NMD on three quarters of the targets that initially appeared to lack premature stop codons. Interestingly, these events occur on non-coding transcript isoforms associated with otherwise normal protein-coding genes. Most of these alternate transcripts are extended at the 5' end and share properties with long un-decoded transcript isoforms (LUTIs), transcripts that initiate from an upstream promoter and suppress a canonical gene promoter. We found that these LUTI-like transcripts encode multiple 5' upstream ORFs (uORFs) that trigger NMD. We also identified a related class of transcripts that initiate transcription within the coding sequence of the gene. Translation of these transcripts occurs on short, out-of-frame ORFs internal to the annotated coding sequence (iORFs) that trigger NMD at associated premature stop codons. Here we show that inhibitory ncRNAs associated with protein-coding genes are a novel class of NMD substrates. Furthermore, NMD is critical for removing these regulatory mRNAs from the cell, perhaps to prevent unproductive translation and the synthesis of potentially toxic peptides. We find that these NMD-targeted ncRNAs are present in many key pathways, including those involved in control of nitrogen and thiamine levels in the cell, suggesting they have widespread regulatory roles.

119A

Understanding the role of J domain and RRM of Cwf23 in RNA splicing

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Cwc23 is an essential J domain protein in *Saccharomyces cerevisiae*, whose J domain, unlike other JDPs, is completely dispensable for its essential cellular functions. It interacts with Ntr1 and is required for its recruitment to the spliceosome during spliceosome disassembly. Spliceosome disassembly requires NineTeen complex-Related (NTR) complex which is composed of Cwc23, Ntr1, Ntr2 and Prp43. Cwc23 being a part of NTR complex, suggests towards an involvement of Hsp70:JDP machinery in regulating spliceosomal dynamics. Our data shows that the C-terminal region of Cwc23 orthologs in higher eukaryotes have acquired novel moonlighting functions over long evolutionary time scales. Cwf23, the Cwc23 ortholog in *Schizosaccharomyces pombe*, presents a fascinating example of protein evolution. The C-terminal domain of Cwf23 has an RRM (RNA Recognition Motif), shows RNA binding activity and is important for interaction with *sp*Ntr1. Additionally, unlike its *S. cerevisiae* ortholog, the J-domain of *S. pombe* protein, Cwf23, is essential for viability. Our results show that in more complex and "intron-rich" eukaryotes, the J-domain and hence the Hsp70 co-chaperone function of Cwc23 orthologs might be more important for RNA splicing. Considering the importance of RNA splicing in eukaryotes, including humans, understanding the role of molecular chaperones in remodeling the spliceosomal complex has immense translational value.

120A

Kar4, the yeast ortholog of Mettl14, is required for mRNA m^{6}A methylation and meiosis

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The mRNA methyltransferase complex is highly conserved across eukaryotes. In mammals, it is composed of Mettl3 (Ime4), Mettl14 (Kar4), and WTAP (Mum2) as well as a host of other regulatory proteins. In yeast, the methyltransferase complex was identified as containing Ime4, Mum2, and Slz1, but not Kar4. In contrast, High throughput studies indicated that Kar4 interacts with both Ime4 and Mum2, raising the question of functional significance of the interaction. We have found that Kar4 is also required for efficient mRNA methylation during meiosis, when levels of the modification are highest. Moreover, we found that Kar4 interacts with both Slz1 and Kfc1, an ortholog of Virilizer, another component of the eukaryotic methylation complex. Like *ime4*Δ, *kar4*Δ mutants are blocked early in meiosis, prior to pre-meiotic S-phase. Over-expression of the early meiotic transcription factor, *IME1*, in a *kar4*Δ mutant rescues an early meiotic transcript defect and permits pre-meiotic S-phase. However, to facilitate sporulation of the *kar4*Δ mutant, over-expression of the translational regulator, *RIM4*, is required in

addition to *IME1*. Using alleles of Kar4 that are specifically defective for either mating or one of its two meiotic functions, we found that all mutants that are proficient for Kar4's early meiotic function maintain some level of interaction with Ime4. Interestingly, over-expression of IME*1*, alone, can rescue the meiotic defect of a catalytically-dead Ime4 mutant. Similarly, *IME1* over-expression is sufficient to suppress slz*1*Δ, which causes a defect in Ime4 and Mum2 nuclear localization. Together, these data suggest that the early function of Kar4 that facilitates entry into and progression through the early stages of meiosis is through nuclear mRNA methylation. The requirement for the over-expression of a translational regulator, *RIM4*, in addition to *IME1,* suggests that the complex may have another post-transcriptional function to facilitate sporulation. Using mass spec, we identified a set of genes required during the later meiotic divisions and spore maturation, which have relatively wild type transcript levels, but which were not detectable at the protein level after *IME1* over-expression. These proteins are expressed upon the additional over-expression of *RIM4.* Given these findings, we hypothesize that the later function of Kar4 that facilitates progression through the meiotic divisions and spore maturation involves ensuring the efficient translation of proteins required at these steps.

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"Terminators at the Terminus": Novel insights into the mechanism of regulation of sub-telomeric transcripts

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Telomeres are specialized nucleoprotein complexes that define the ends of the chromosomes. Transcription of genes located next to the telomeres is usually kept repressed by a phenomenon called telomere position effect (TPE). This regulation is achieved via epigenetic silencing and any leaky transcripts from the telomeres are post-transcriptionally degraded by RNA surveillance pathways. But the longstanding idea that telomeric regions are transcriptionally repressed has to be revisited because of the current discovery of TERRA (telomeric repeat-containing RNA), ARRET, sub-telomeric XUTs, sub-telomeric CUTs and several other RNA species which originate from telomere ends. These RNA molecules remain associated with the telomeric chromatin suggesting a potentially regulatory role in telomere replication and architecture. The sub-telomeric regions also show higher levels of recombination leading to faster evolution of gene families residing in this locus. This allows faster and better adaptive responses to the changing environment. Additionally, it contributes to antigenic variation and virulence in some pathogenic yeasts and parasites. Therefore, understanding regulation of telomeric and sub-telomeric transcription has implications beyond yeast.

Our work in *Saccharomyces cerevisiae* indicates that Rtt103 - a transcription termination factor, plays a prominent role in sub-telomeric silencing. *rtt103*Δ is defective in TPE with increased levels of sub-telomeric and TERRA transcripts. This is also observed in the case of *rai1*Δ and *rat1-1,* which are postulated to work with Rtt103 in transcription termination. Further we demonstrate that the recruitment of Rat1 to the telomeres occurs via Rtt103 in a transcription-dependent manner. Our findings indicating the novel role of Rtt103 and its partners in telomere biology will be presented.

Keywords: **Telomeres, TPE, TERRA, Rtt103**.

122A

Transcriptional Regulation by Histone H3 Lysine 4 Methylation in *S. cerevisiae*

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Transcription is an essential process, required for the production of proteins and non-coding RNAs in all organisms. In eukaryotic cells, DNA is wound around histone proteins forming chromatin that impacts transactions involving genomic DNA. A dynamic form of transcriptional regulation involves the interplay between the writers and readers of the post-translational modifications on histone proteins. My research focuses on understanding how methylation of histone H3 in chromatin regulates transcription by RNA polymerase II. Set1 is a writer, a histone methyltransferase that catalyzes mono, di and tri-methylation of the epsilon amino group of the fourth lysine on the N terminal tail of histone H3 (H3K4). Previous research has shown that mono, di and tri methyl marks on H3K4 act in discrete ways to regulate transcription. The concept that modification of the same amino acid residue in a histone can have profoundly different effects on transcription is intriguing and requires further investigation. To gain insight into how methylation of H3K4 contributes to the regulation of transcription, variants of the Set1 protein that differentially methylate H3K4 are being studied. Mutants of *set1*, null for H3K4 methylation, exhibit growth defects during histidine starvation or isoleucine/valine starvation. These growth defects are rescued by either a wild-type allele of *SET1* or partial-function alleles of *set1* that express variants capable of mono methylation or mono- and dimethylation of H3K4. The *set1* null mutants make less *HIS3* and less *ILV6* mRNA than a wild-type *SET1* strain. To identify other genes in yeast that are potentially regulated by H3K4 methylation, RNA sequencing experiments were performed using 3 amino triazole (3AT) to induce nutrient stress. Gene ontology analysis of the RNA-seq data revealed that genes involved in amino acid metabolism, cell wall organization, sporulation and meiosis were either positively or negatively impacted by changes in the degree of H3K4 methylation. New hypotheses on gene regulation based on the RNA-seq results will be discussed. Set1-like H3K4 methyltransferases are evolutionarily conserved and have been linked to developmental gene regulation in higher eukaryotes. Identification of H3K4 methylation-mediated gene regulatory mechanisms in yeast are likely to lead to better understanding of complex regulatory processes in higher organisms.

123A

Predicting yeast transcriptional activation domains from amino acid sequences

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Transcription factors serve the important function of activating gene expression by using DNA binding domains and activation domains. While DNA binding domains fold into 3D structures and are conserved, activation domains are intrinsically disordered and not conserved. Thus, DNA binding domains can be predicted from an amino acid sequence while activation domains remain difficult to predict. Using a human activation domain predictor as a model, we built an altered yeast activation domain predictor. We decided to use yeast as a model system in order to study transcription factor evolution and activation domain evolution. Since aromatic residues are important in yeast activation domains and hydrophobic residues such as leucine are important in human activation domains, the yeast model focuses on different residues than the human model. We will use this yeast activation domain predictor to study the conservation of activation domains across orthologous transcription factors from diverse yeast species.

124A

Monte Carlo simulations of GCN4 orthologs reveal features of transcriptional activation domains that confer high and low activity

Angelica Lam, Jordan Stefani, Melvin Soriano, Max V Staller Center for Computational Biology, University of California, Berkeley

Activation domains of transcription factors regulate gene expression by binding to coactivators to activate transcription, but it is difficult to understand what sequence features control their activity because they are intrinsically disordered and poorly conserved. Previous findings in GCN4 have proposed that acidic residues of activation domains keep hydrophobic residues exposed to solvent for interactions with coactivators, suggesting that strong activation domains in yeast can be identified as highly acidic regions interspersed with aromatic residues. However, 40-amino acid regions of GCN4 orthologs with these sequence features demonstrate both high and low activation domain activity. To understand the structural features that separate high and low activity, we ran all-atom Monte Carlo simulations of these disordered regions. By simulating their conformational ensembles, we can analyze how exposure of hydrophobic residues correlates to highly active regions. Findings from the simulations will ultimately inform a predictor of activation domains from protein sequences.

125B

Meiotic Regulation of the Unfolded Protein Response

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Successful gamete formation depends on faithful execution of meiosis via a highly coordinated developmental program. Recent work has identified timed induction and silencing of the conserved endoplasmic reticulum (ER)-localized unfolded protein response (UPR) during late stages of budding yeast meiosis, suggesting a role in gamete formation for this conserved cellular stress response. Decreased sporulation efficiency and spore viability were observed as a result of meiotic HAC1 or IRE1 depletion. Drug induced UPR is known to rely on the lumenal domain (LD) of IRE1 to sense unfolded proteins, while it is unclear if meiotic UPR induction would similarly need to sense unfolded proteins via the LD. We are currently employing a variety of genetic and cell biological techniques to gain insights into developmental UPR activation and its relevance to meiotic success.

126B

The hunt for the promoter element controlling Whi5's size-independent transcription

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Cells regulate their size to an optimal range to maintain functionality and fitness. They achieve this by coupling cell growth to cell division. Smaller-born cells grow more during G1 before entering the cell cycle to correct for their initially smaller size. Previous work in our lab identified the G1/S transcriptional inhibitor Whi5 as a key regulator of this cell size homeostasis in budding yeast. Whi5 binds the master G1/S activator SBF and thereby inhibits expression of G1/S transition genes. Crucially, Whi5 is diluted throughout G1, thereby allowing cells to "sense" the increase in cell volume. As cells increase in size in G1, Whi5 is diluted and its inhibition of SBF is weakened so that cells are more likely to enter the cell cycle.

For this "inhibitor dilution" model to work, cells must be born with similar amounts of Whi5. This is achieved through a combination of association with chromatin before cytokinesis and a size-independent synthesis rate during S/G2/M. However, the mechanism behind size-independent synthesis of Whi5 is unknown. While most protein synthesis rates scale with cell size to maintain consistent concentrations, Whi5 synthesis rates do not increase with cell size. We have established that this is regulated at transcription. *WHI5* mRNA amounts are also size-independent and promoter swap experiments show that the *WHI5* promoter is both necessary and sufficient for size-independent synthesis.

Here, we present ongoing work to determine the mechanism behind WHI5's size-independent transcription. We have generated a series of strains harboring short deletions throughout the WHI5 promoter. We have then quantified the scaling behavior of Whi5-mCitrine expressed from of each of these mutant promoters by single-cell time-lapse microscopy. This has uncovered a 91bp region in the WHI5 promoter that when deleted weakens sub-scaling, increasing the expression of Whi5 in larger cells. Once we isolate a small enough region responsible for

size-independent transcription, we can employ a yeast 1-hybrid screen to isolate candidate transcription factors that drive this size-independent transcription mechanism.

127B

Investigating the role of the Spt5 C-terminal repeat domain and its phosphorylation in transcription regulation

Sanchirmaa Namjilsuren, Karen Arndt Biological Sciences, University of Pittsburgh

Transcription elongation by RNA polymerase II (Pol II) is a dynamic and highly regulated step in the gene expression cycle that involves the participation of multiple accessory factors. Transcription elongation factors form an elongation complex with Pol II and help coordinate chromatin states with transcript synthesis. Among these factors, Spt5 is an essential and highly conserved factor that is known to maintain Pol II processivity, regulate promoter-proximal pausing in metazoans, and couple chromatin modification states and co-transcriptional RNA processing to elongation. Some important functions of Spt5 are facilitated by its C-terminal repeat domain (CTR) in a phosphorylation-dependent manner, which is reminiscent of gene expression control by the Pol II CTD. Previous work from our lab and others has shown that the phosphorylated Spt5-CTR is required for proper assembly of the Pol II elongation complex via recruitment of the Polymerase Associated Factor 1 Complex (Paf1C). The CTR is a conserved feature of Spt5, however it is dispensable for the viability of yeast cells, and the mechanistic impact of CTR phosphorylation or dephosphorylation in transcription is not well understood. In this study, we sought to comprehensively identify the genetic interactors and binding partners of the CTR using unbiased genetic and proteomic screens, respectively. SAturated Transposon Analysis in Yeast (SATAY) assay identified candidate genes, involved in various transcription-associated processes such as mRNA processing and chromatin remodeling, that show negative or positive genetic interactions with the CTR. For example, the deletion of the CTR suppresses the growth defect of a yeast strain lacking Ref2, a component of cleavage and polyadenylation factor. A site-directed crosslinking experiment revealed proteins that specifically interact with the CTR *in vivo*. Together, our results are identifying molecular pathways that require a functional CTR and proteins that directly interact with the CTR, thus providing insights into another layer of transcription regulation.

128B

One carbon metabolic enzymes and cell cycle progression

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Folate-based, one-carbon (1C) metabolism encompasses the chemical reactions that move and use single-carbon functional groups (one-carbon units). 1C pathways are directly involved in the metabolism of amino acids (primarily Ser, Gly, Met, and His) and the synthesis of purines, thymidylate, and phospholipids. As a result, 1C outputs govern vital cellular processes, including genome replication and maintenance (through nucleotide synthesis), response to oxidative stress (through glutathione synthesis), and gene expression (through methylation of DNA and histones), among others. Recently, we reported that the translational efficiency of several 1C enzymes changes in the cell cycle. Furthermore, the corresponding loss-of-function mutants had increased replicative longevity. To follow up on these findings, we examined the cell cycle kinetics of these mutants and measured the protein abundance of several 1C enzymes in the cell cycle. We found that the abundance of Ade17p, an enzyme of 'de novo' purine biosynthesis, peaked strongly late in the cell cycle. Furthermore, while cells lacking Ade17p grow in size at the same rate as wild-type cells, they have a larger critical size, consistent with a delay at Start, when cells commit to a new round of cell division. We also found that the role of Ade17p in longevity (*ade17* yeast mutants are long-lived) may be conserved in other organisms since chemical inhibition of Ade17p increases *C. elegans* lifespan. Our results suggest that 1C metabolism impacts cell cycle progression in multiple but distinct ways. Adjusting the output of 1C pathways may also be a conserved route to improved longevity.

129B

Genome-wide Analysis of the Transcriptional Response of Wild-type and adn2Δ Cells in Response to Perturbation of the Cytokinetic Machinery

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In the fission yeast, Schizosaccharomyces pombe, treatment with low doses of the actin depolymerizing drug Latrunculin A (LatA) results in a delay in cell cycle progression and the prolonged maintenance of a cytokinesis competent state. In this state, fission yeast cells are able to continuously reform/repair the actomyosin ring until cell division is successful. To identify genes that play a role in this response, a genome-wide library of S. pombe gene deletion mutants was previously screened for hypersensitivity to LatA. While the largest group of identified genes comprised regulators of cytoskeletal dynamics, the second largest group comprised regulators of transcription. Among the transcriptional regulators identified, the LisH domain containing transcription factor adn2 (adhesion defective protein) showed one of the strongest phenotypes in response to LatA and was thus characterized further. Interestingly, live-cell imaging experiments demonstrated that adn2 gene deletion mutants, while initially able to assemble the actomyosin ring, were unable to properly constrict the ring in the presence of LatA leading to cell division failure and the eventual formation of abnormal tetra-nucleate cells. Furthermore, over-expression experiments demonstrated that abnormally high levels of Adn2p result in morphological/cytokinetic phenotypes suggesting a possible dominant-negative effect. PCA analysis of gene expression profiles derived from a time-course of LatA treated cells, revealed that while adn2Δ samples remained clustered irrespective of LatA treatment, wild-type samples exhibited a structured co-expression in response to LatA resulting in distinct clusters in the PCA plot. Consistent with this analysis, a total of 888 genes were found to be differentially expressed in wild-type cells (624 up, 264 down), while only 243 genes were differentially expressed in adn2Δ mutants (89 up, 154 down). Furthermore, analysis of the differentially expressed genes identified pathways involved in the "cellular response to toxic substances" as significantly enriched in LatA treated wild-type cells, but not in similarly treated adn2Δ cells. These data suggest that Adn2p mediated changes in gene-expression in response to cytoskeletal perturbations are important in maintaining the fidelity of cytokinesis in fission yeast.

130B

Modularization, minimization, and diversification of the yeast transcription factor repertoire

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Chromosome synthesis can be used to reorganize genomes in ways that help improve understanding of cellular life and evolution. Here, we are engineering a yeast strain that can be used to better explore global transcriptional control and its relationship to phenotypic diversity. We are relocating the roughly ~200 DNA binding, RNA polymerase II-associated transcription factors (TFs) in Saccharomyces cerevisiae into a single functional module on a neochromosome. The TF neochromosome is being synthesized by assembling ~260 gene-sized pieces of synthetic DNA into a single molecule. We are also constructing TF-free native chromosomes through cloning and reassembly of TF-free natural DNA segments. The outcome of this work will be a cell in which nearly all transcriptional regulation is controlled by a synthetic module on a distinct chromosome. This module will provide a platform for probing minimal sets of TFs required for viability. A minimal TF cohort will then enable explorations of the phenotypic diversity achievable through the reintroduction of accessory TFs.

131B

The transcription elongation factor Rtf1 regulates distribution of the nucleosome remodeler Chd1 on active genes

Sarah Tripplehorn, Hannah Marvil, Sarah Hainer, Karen Arndt University of Pittsburgh

Nucleosomes impede DNA-templated processes and enforce transcriptional fidelity by preventing transcription from aberrant start sites. The nucleosome remodeler Chd1 dynamically controls nucleosome positioning genome-wide, but it is unclear how Chd1 function is regulated and whether Chd1 recruitment to chromatin is related to the transcription cycle. Our lab previously found that the transcription elongation factor Rtf1, a member of the Paf1 Complex (Paf1C), is able to interact with Chd1 and is also partly responsible for Chd1 occupancy on chromatin. Rtf1 is also required for the co-transcriptional deposition of histone modifications and contributes to Paf1C recruitment to the RNA Polymerase II transcription elongation machinery. Through site-directed mutagenesis and yeast two-hybrid experiments, we have localized the interacting regions of Rtf1 and Chd1 and designed point mutations in Rtf1 that disrupt the Chd1 interaction, while maintaining other functions of Rtf1. Through chromatin immunoprecipitation (ChIP) experiments, we found that the C-terminus of Chd1, an understudied domain, is required for occupancy of Chd1 on chromatin. Furthermore, the N-terminal region of Rtf1 is required for proper distribution of Chd1 along gene bodies. Point mutations in *RTF1* that disrupt the interaction with Chd1 lead to cryptic intragenic transcription, demonstrating the importance of this interaction for proper transcriptional control. Currently, we are addressing the impact of the Rtf1-Chd1 interaction on global nucleosome positioning using separation-of-function mutations in *RTF1* and micrococcal nuclease (MNase)-sequencing. Consistent with the high conservation of these two protein domains in mammals, the Rtf1-Chd1 interaction may also occur in mammals, and we are currently designing constructs to test this interaction in mouse embryonic stem cells. Together, these data indicate that Rtf1 and Chd1 function together during transcription to facilitate nucleosome remodelling in yeast.

132B

Investigating transcriptional interference induced by long undecoded transcript isoform (LUTI) expression

Kate Morse, Sarah Swerdlow, Elçin Ünal UC Berkeley

Cellular differentiation is driven by changes in gene expression. To transition from one type of cell to another, cells must simultaneously activate genes that promote differentiation while repressing genes that antagonize faithful progression. How gene repression is achieved amidst widespread transcriptional activation is not fully understood. A recently described mode of gene repression, which relies on integrated transcriptional and translational interference, exemplifies the coordination of transcriptional activation and gene repression. In this form of gene regulation, a long undecoded transcript isoform (LUTI) is transcribed from a gene-distal promoter, interfering with expression of the gene-proximal promoter. In contrast to the efficiently translated, canonical transcript derived from the proximal promoter, the LUTI contains upstream open reading frames in its 5' extended sequence which prevent translation of the protein coding sequence.

First observed in budding yeast gametogenesis - the differentiation program required to produce sex cells - and later during the yeast unfolded protein response and in human cells, LUTI-based regulation allows cells to toggle between a repressive and coding transcript throughout differentiation. Furthermore, LUTI-dependent interference over the proximal promoter is tunable and reversible, permitting dynamic gene expression upon cellular state changes, as in stress response or development. While transcription and co-transcriptional chromatin modifications induced by LUTI expression have been described, trans-acting factors required for LUTI-dependent gene repression have yet to be uncovered. Using an unbiased genetic approach, we have found that subunits of the SWI/SNF chromatin remodeling complex are required for co-transcriptional chromatin changes that lead to silencing of the proximal promoter derived, coding transcript. Thus, we have uncovered a novel repressive activity by the SWI/SNF complex through this unconventional mode of gene regulation.

133B

Genetic dissection of transcription start site selection in *Saccharomyces cerevisiae*

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Transcription initiation by RNA Polymerase II necessitates involvement of general transcription factors together with the 12-subunit Pol II complex to form the pre-initiation complex (PIC). In *Saccharomyces cerevisiae*, PICs scan the promoter downstream from an initial

recruitment site to identify transcription start sites (TSSs). Mutations affecting this process have been found to shift TSS usage distributions upstream or downstream at the majority of yeast promoters. We have identified multiple examples of mutations within the same factor having the opposite effect on TSS distributions, suggesting TSS selection can be plastic and is highly sensitive to factor activity. Previous approaches have only likely identified a subset of PIC components whose activity can control TSS selection. We are leveraging unbiased genetic selections to identify the range of factors and surfaces within the PIC that control TSS selection. These genetic selections deploy both novel and existing initiation reporters to identify TSS-shifting mutants. Individual reporters can detect either upstream or downstream shifting mutants, and we have isolated mutants belonging to each "directional" class. We have employed a targeted amplicon sequencing approach to identify candidate TSS selection mutants among a curated set of ~300 yeast genes linked to transcription or chromatin control. Our preliminary selections have identified both novel and previously-studied mutations, thereby authenticating our method. We have identified three main phenotypic profiles that correspond to "chromatin architecture regulating factors", "promoter scanning assisting factor", "polymerase catalysis class", among possible others. We have engineered our strains to increase throughput for complementation tests by incorporation of appropriate selectable markers. Furthermore, we are creating a battery of marked TSS selection mutants to allow for parallel genetic interaction studies with identified candidate alleles. Specific genetic interactions enable prediction for where candidate alleles may be defective in the TSS selection process. Our system will reveal the network of factors that participate in TSS selection in *S. cerevisiae*.

134B

The decrease in mRNA decay as cell size increase to compensate for non-linear transcriptional scaling Crystal Tsui Bioengineering, Stanford University

It is observed in cellular growth that as cell size increases, mRNA and protein amounts also increase to maintain a relatively constant concentration in the cell. This scaling behavior allows the cell to function normally as the cell grows and increases in size. For example, in cases where biosynthesis breaks down in abnormally large cells, cellular functions including growth and induction are impacted (Neurohr et al.).

In Swaffer et al., RNA polymerase II amount is found to be the limiting factor of transcription, and that as cell size increases, transcription does not scale proportionally to cell size. As transcription lags in larger cells, the non-linear deviation should theoretically impact the concentration of mRNA. However, as mentioned already, mRNA concentrations seem to be relatively constant with respect to cell sizes. This suggests a mechanism that keeps mRNA concentrations constant when transcription lags behind. Hence, we propose a model where mRNA concentration is maintained in larger cells through lower mRNA decay rates. In order to validate the model, we repressed the transcription of endogenous genes using two separate systems, the MET and GAL system, in S.cerevisiae that is induced to different sizes. We measured the particular mRNA transcript levels through RT-qPCR and calculated the decay rates for the mRNA transcripts. We found that the mRNA decay rate decreases as cell size increases, matching the proposed model. We also aim to measure the global mRNA decay rates for different cell sizes through 5EU pulse-chase labeling followed by sequencing.

The decrease of mRNA decay rates as cell size increases allows mRNA concentration to stay stable despite a non-linear scaling of transcription with cell size, which in turn aids the scaling of protein with cell size. The specific mechanism of this control of mRNA decay rates is still unclear but it is likely a feedback mechanism that is not directly controlled by size.

135B

Differential regulation of the mitotic G1/S transcriptome is required for timely meiotic entry Amanda J Su, Elçin Ünal MCB, University of California, Berkeley

Transcription factors induce dynamic changes in gene expression to drive cellular differentiation. During the G_1/S transition in the cell cycle of budding yeast, transcription factors SBF(Swi4-Swi6) and MBF(Mbp1-Swi6) play essential roles in cellular commitment to mitotic division. While SBF and MBF act in parallel to mediate the mitotic G1/S transition, there is an observed functional separation of SBF and MBF targets. We hypothesize that the differential regulation and proposed specialized function of meiotic SBF and MBF are necessary for proper expression of the meiotic program. Unlike mitotic G_1/S, during meiotic entry Swi4 decreases in protein and nuclear abundance as SBF target expression generally decreases. We found this decrease in Swi4 protein abundance in part to be regulated by expression of a repressive mRNA isoform that results in transcriptional and translational interference. To investigate if Swi4 abundance is important for establishing the meiotic G_1/S transcriptome we overexpressed Swi4 and found that overexpression is sufficient to turn on expression of SBF targets in meiosis. In addition to the repression of SBF target expression in meiosis, transcription factor Ime1 is nuclear and increases in expression as cells enter meiotic G_1/S. However, we found that upon overexpression of Swi4 in meiosis fewer cells have nuclear Ime1 as well as decreased expression of early meiotic genes namely many targets of Ime1. Taken together, these data suggest SBF target expression needs to be regulated in part by shutting off Swi4 protein expression for timely expression of early meiotic transcriptome by Ime1.

136B

Environmental Effects on Transcriptional Activation Domain Function Jordan Stefani[1], Melvin Soriano[1], Max V Staller[21] Center for Computational Biology, University of California, Berkeley, [2]Center for Computational Biology, UC Berkeley

Transcription factors contain effector domains which regulate gene expression. Activation domains are a type of effector domain that interacts with coactivators to upregulate transcription. Activation domains often reside in disordered and poorly conserved regions of transcription factors, making traditional sequence-based methods unsuitable for their characterization. We use high throughput methods to study the protein sequence features that control activation domain function. Since little is known about environmental effects on activation

domain behavior, we are screening for changes in domain function caused by environmental perturbations. While our current FACS-based system for screening activation domains allows us to measure activation strength, we are also developing a new RNA-based high throughput method for screening activation domains in yeast. We anticipate this new method will allow us to assess both speed and strength of activation over time.

137B

Examining the evolution of yeast transcription factor activation domains

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An activation domain is a part of a transcription factor that is responsible for increasing the probability of a gene being transcribed from DNA to mRNA. These domains bind to other proteins known as coactivators, which recruit RNA polymerase II. However, despite many activation domains having the same function, they are poorly understood because of their lack of sequence conservation and intrinsic disorder. Previous work on the Gcn4 and Gal4 transcription factors has identified hydrophobic and acidic amino acid residues that contribute to activation domain function. We searched 207 yeast genomes for orthologs of Gcn4, identifying 502 unique proteins. We found that activation domains are much less conserved than DNA binding domains. Alignments of these orthologs show that some, but not all, of the hydrophobic residues important for activity are conserved. The acidic residues were not well conserved, but we found evidence for interconversion of negatively charged residues near important hydrophobic motifs. We show how functional and structural constraints shape the evolution of the Gcn4 and Gal4 activation domains.

138B

Engineering Synthetic Transcriptional Activation Domains from Conserved Sequence Properties

Melvin Soriano, Jordan Stefani, Sanjana Kotha, Angelica Lam, Giovani Pimentel-Solorio, Ananya Bahugudumbi, Max V. Staller Center for Computational Biology, University of California, Berkeley

Transcription factors bind DNA with DNA binding domains and activate transcription with transcriptional activation domains. Synthetic transcription factors with engineered DNA binding domains and viral activation domains have become useful for synthetic biology, transgenic plants, CRISPR-activation, and have the potential to transform gene therapy. It is now possible to target synthetic transcription factors to nearly any locusi, either with synthetic DNA binding domains or with Cas9. By comparison, there are very few validated activation domains for synthetic biology. To generate a toolbox of synthetic activation domains with different strengths, we are designing activation domains based on libraries of known active activation domains, predicting their activity, and then experimentally determining their activity with a high-throughput yeast assay. We plan to use these data to improve machine learning models for predicting activation domains from protein sequences. These synthetic activation domains will be used for genome engineering and building transgenic plants.

139V

Dominant effects of the histone mutant H3-L61R on Spt16-gene interactions in budding yeast

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The highly conserved FACT complex plays key roles in a variety of chromosomal processes including transcription elongation. During elongation, FACT – which in *S. cerevisiae* is composed of two proteins, Spt16 and Pob3 – contributes to the disassembly of nucleosomes in front of RNA Polymerase II (Pol II) and to their reassembly in the wake of Pol II passage. In previous work we identified a nucleosomal region – termed ISGI (Influences Spt16-Gene Interactions) – that plays an important role in promoting proper interactions between yeast FACT (yFACT) and transcribed genes. More specifically, mutations within this region cause a shift in yFACT occupancy toward the 3' ends of genes, an effect we attribute to impaired yFACT dissociation from genes following transcription.

Two recent studies have unveiled an association between a mutation within one of the two genes that encode the human histone H3.3 protein and neurodevelopmental disorders in patients. This mutation results in a substitution of the ISGI residue L61 to an arginine (H3.3-L61R), and, if this mutation is indeed responsible for the disease phenotype, it must act in a dominant fashion since the genomes of these patients also harbor three alleles encoding wild type histone H3.3. Our previous work in yeast has shown that most substitutions at H3-L61 cause strong defects in Spt16-gene interactions, but the H3-L61R mutant had not been tested in those studies due to the fact that it does not sustain viability when expressed as sole source of histone H3 in cells. In the present study, we tested H3-L61R for possible dominant effects in conferring defects in Spt16-gene interactions and found that it not only impairs proper Spt16 dissociation from genes in a dominant fashion, but it does so also in cells that express the mutant protein from one of four histone H3-encoding alleles. These results, combined with other studies linking loss of function mutations in human Spt16 and neurodevelopmental disorders, provide a possible molecular mechanism underlying the neurodevelopment disorders seen in patients expressing the histone H3.3-L61R mutant.

140V

Understanding the Role of Cdk8 in Glycolysis Gene Regulation

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Transcription regulation is an essential molecular process that organisms use to respond to internal and external cues. When transcription is dysregulated, it can result in a variety of disease states, underscoring the need to understand the underpinnings of normal transcriptional

processes. A key player in transcriptional regulation is the cyclin dependent kinase 8 (Cdk8). Cdk8 regulates transcription through a variety of mechanisms, including the regulation of RNAPII-Mediator complex interactions, the phosphorylation of RNAPII, and the modulation of transcription factor activity via phosphorylation. Highlighting a role in human health, Cdk8 functions as an oncogene in colorectal cancer, a role that involves effects on the transcriptional regulation of glycolysis genes. However, the molecular mechanisms by which Cdk8 regulates glycolysis remains unknown. Work currently under review from our laboratory describes a new mechanism by which Cdk8 regulates glycolysis gene expression in *Saccharomyces cerevisiae,* one that involves the phosphorylation of Gcr2, a transcriptional activator of genes encoding glycolysis enzymes. This work has also identified Gcr2-S365 as a site for Cdk8-dependent phosphorylation and suggested that phosphorylation at additional sites may contribute to glycolysis gene expression regulation. My research will identify all amino acid residues on Gcr2 that are phosphorylated by Cdk8 and determine the mechanisms by which Cdk8-dependent phosphorylation regulates glycolysis gene expression regulation.

To study the effect of Cdk8-dependent phosphorylation on Gcr2 and glycolysis gene expression regulation, I have generated a library of *GCR2* phospho-mutant and phosphor-mimic alleles that affect the phosphorylation state of Gcr2 at S365 and S247 a putative site of Cdk8-dependent phosphorylation. Importantly, all the *gcr2* alleles generate normal levels of Gcr2 protein, suggesting that they do not result in the generation of unstable proteins. These alleles will be subjected to a battery of assays, including RT-qPCR to examine changes in glycolysis gene mRNA levels. Ultimately, this work will reveal how Cdk8 regulates Gcr2 and glycolysis gene expression, identifying new pathways by which Cdk8 regulates transcription and a potential mechanism by which Cdk8 contributes to dysregulated glycolysis in colorectal cancer cells.

141V

Expression of defective human *SHQ1* variants in yeast decreases the stability of H/ACA small nucleolar RNAs and impairs ribosome biogenesis

Ismaël Alidou-D'Anjou, Aniket Patel, Sophie Sleiman, François Dragon Sciences biologiques, University of Quebec at Montreal

SHQ1 is a conserved chaperone that binds the pseudouridine synthase dyskerin in the cytoplasm, and escorts the enzyme to the nucleus. Dyskerin must assemble with other nuclear factors to form H/ACA small nucleolar ribonucleoproteins (snoRNPs). These abundant particles carry out pseudouridine formation in ribosomal RNAs and participate in maturation of rRNA precursors. Compound heterozygous mutations in *SHQ1* have been linked to a severe neurological disorder, and *in vitro* studies with bacterially expressed proteins indicated that each mutation weakened the interaction of SHQ1 with dyskerin (1).

We generated a conditional yeast strain that expresses endogenous, chromosome-encoded Shq1 under the control of the *GAL1* promoter, allowing cellular depletion of Shq1 protein by shifting cells from galactose- to dextrose-containing medium. Growth in dextrose is lethal because *SHQ1* is an essential gene; however, cells can be rescued by constitutive expression of plasmid-borne FLAG-tagged Shq1 protein (2). Using this system we carried out complementation studies with human *SHQ1* variants (1), and examined how they affect cellular growth. Unlike wild-type human SHQ1, variant p.R335C could not sustain growth in dextrose-containing medium, while variant p.A426V showed temperature-sensitivity and cells could not grow at 37 °C. Western blotting analyses indicated that Cbf5 (the yeast homolog of dyskerin) was unstable in restrictive conditions. Immunoprecipitation experiments were done with cellular extracts prepared from cells expressing FLAG-tagged SHQ1 variants: those experiments revealed that co-immunoprecipitation of Cbf5 was less efficient with SHQ1 variants compared to wild-type SHQ1, in line with previous pull-down experiments (1) and our yeast two-hybrid assays. Northern hybridization analyses indicated that H/ACA snoRNAs were largely unstable in cells expressing SHQ1 variants in restrictive conditions; in contrast, levels of the abundant C/D snoRNAs (required for 2'-O-methylation of rRNAs) were unaltered. These changes in abundance of H/ACA snoRNAs severely impacted rRNA maturation and the formation of ribosomes.

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142A

The yeast Ty1 retrotransposon contains a prion-like domain essential for transposition

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The cytosolic foci that nucleate virus-like particle (VLP) assembly in the yeast LTR-retrotransposon Ty1 are not well-understood. These foci, termed retrosomes or T-bodies, contain Ty1 Gag and likely Gag-Pol and the Ty1 mRNA destined for reverse transcription. Here, we propose the retrosome is a liquid-liquid phase separated (LLPS) compartment that concentrates the constituent building blocks of VLPs and is required for retromobility. Membrane-less compartments are observed in viral life cycles, including viral factories, have been reported in the retrovirus HIV-1 driven by nucleocapsid protein, and recently, the human LINE-1 retrotransposon ORF1 protein was shown to phase separate *in vitro*. LLPS is, in part, driven by unstructured, intrinsically disordered protein sequences that facilitate aggregation and demixing from the surrounding environment. Also, protein aggregation is associated with a growing list of human diseases including neurodegeneration and prion diseases. Prions have been found in all branches of life, including viruses.

The case for LLPS in Ty1 biology is supported by our discovery of an intrinsically disordered N-terminal prion-like domain (PrLD) within Gag that is required for transposition. This domain contains amino-acid composition similar to known yeast prions and is sufficient to nucleate prionogenesis in an established Sup35 prion reporter system. Deleting the Ty1 PrLD results in dramatic VLP assembly and retrotransposition defects but does not affect Gag protein level. Ty1 Gag chimeras in which the PrLD is replaced with other sequences,

including yeast and mammalian prionogenic domains, display a range of retrotransposition phenotypes from wildtype to null. We examine these chimeras throughout the Ty1 replication cycle and find that some support VLP assembly and retrotransposition, including the yeast Sup35 prion and the mouse PrP prion. We are investigating the role of the Gag PrLD in retrosome formation and determining what characteristics of the PrLD constrain function in Ty1. Our yeast-based system may provide a useful, genetically tractable, *in vivo* model for studying LLPS, complete with a suite of robust and sensitive assays, and host modulators developed to study Ty1 retromobility. Our work invites study into the prevalence of phase separation in additional retroelements.

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Modeling the fitness cost of chromosomal duplication identifies determinants of aneuploidy toxicity

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Whole chromosome aneuploidy, in which cells carry an abnormal number of chromosomes, affects growth and fitness across most eukaryotes. But what causes aneuploidy toxicity at a cellular level remains poorly understood. One model is that aneuploidy leads to proteome imbalance that causes proteostasis stress. In *Saccharomyces cerevisiae*, the consequences of aneuploidy have been best characterized in the lab strain W303, which is particularly sensitive to chromosome duplication. In contrast, wild isolates are more tolerant of chromosome amplification and often display some level of aneuploidy in nature. Therefore, the true nature of aneuploidy stress remains unresolved. To study the mechanisms through which chromosome duplication impacts fitness, we systematically characterized the fitness costs of duplicating each of the 16 yeast chromosomes in the wild oak-soil isolate YPS1009. We then computationally modeled fitness costs, testing separable biological hypotheses, to understand the determinants of aneuploidy toxicity. The distribution of measured fitness costs was relatively well explained by simply summing the number of genes per chromosome, consistent with the hypothesis that additional burden in genes expression affects fitness independently of gene functions. The data were similarly explained by the additive contributions of transcript or protein levels. However, these models do not consider the specific properties of genes amplified on those chromosomes. To explore the impact of specific amplified genes, we used a low copy plasmid library to measure fitness effects of genes duplicated individually. We then modeled the fitness cost of each chromosome duplication based on the additive cost of genes duplicated on that chromosome. Accounting for individual gene toxicity significantly improved the model, explaining 80-85% of the variance in aneuploidy fitness costs. The gene-overexpression analysis also identified genes that improve fitness. Interestingly, discounting the contribution of these beneficial genes decreased the explanatory power of the model, suggesting that some amplified genes lower the fitness cost of specific chromosome duplications. Our results indicate that aneuploidy toxicity comprises both gene-independent contributions to proteostasis burden and gene-specific impacts for which both deleterious and beneficial impacts matter. The latter points to toxic genes that drive aneuploidy stress and may provide a target through which to alleviate aneuploidy toxicity.

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A CRISPRi-based approach of identifying and quantifying fitness impacts of Hsp90 client-chaperone interactions.

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The maintenance of a functional proteome relies upon the activity of molecular chaperones. Chaperones interact with and participate in the refolding of proteins following their delivery by interacting proteins called co-chaperones. Hsp90 is a chaperone that is conserved from bacteria through humans and participates in the folding of newly synthesized, misfolded proteins and the assembly of protein complexes. A critical issue central to understanding the contribution of Hsp90, and more generally chaperones as a whole, is the identification of their protein clients. We present an approach that aims to identify the protein clients of Hsp90 in a systematic approach. Our method involves a CRISPRi knockdown of two Hsp90 cochaperones in yeast, Cdc37 and Ydj1 which are responsible for the delivery of proein clients to the chaperone. We follow the impact of knocking down either of these co-chaperones in a library where the majority of yeast genes are tagged at their C-terminus with murine dihydrofolate reductase (mDHFR), which confers resistance to methotrexate. Using this library we follow the fitness of individual strains across conditions to identify proteins whose abundance and corresponding fitness are disrupted by the CRISPRi knockdown of either co-chaperone. We discuss our results here and the general utility this approach may offer to the study of chaperones and additional systems that impact protein abundance.

145A

Protein dynamics of whole-genome duplicates using single-cell imaging and automated analysis reveals mechanisms of gene retention

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Gene duplication is pervasive in eukaryotes and is thought to contribute to genomic robustness. While transcriptional and protein-interaction rewiring of the compensating paralogs has been widely studied, the compensation through subcellular relocalization of paralogs has remained largely unexplored. In this study, we used an automated form of yeast genetics, called Synthetic Genetic Array (SGA) analysis coupled with a high content screening of paralog pairs from the GFP collection to delineate the protein dynamics of paralogs in response to

the gene deletion of the corresponding sister paralog. We interrogated 90 paralog pairs derived from the whole-genome duplication by generating a total of 360 paralog-GFP strains in wild-type and deletion backgrounds of the corresponding sister paralog, which exhibit divergent subcellular localization patterns. We used a machine learning-based automated image analysis for segmentation and feature extraction. We then applied dimensionality reduction of the imaging data using Principal Component Analysis (PCA) to quantify the subcellular relocalization and protein abundance responsiveness of the paralogs. The comparison of paralog-GFP in backgrounds with wild-type versus deletion status of the sister paralog revealed that subcellular localization changes were less frequent than protein abundance changes. Cases of compensation, in which the remaining paralog is responding by changing its subcellular localization to compensate for the deleted sister paralog or showing an increase in its protein abundance, were as prevalent as a dependency, loss of the paralog's subcellular localization, or protein abundance in the context of their sister paralog. Responsive paralogs correlated with evolutionary and physiological features, such as protein sequence divergence and the degree of protein-protein interactions. The compensation achieved by subcellular localization changes involved mechanisms consistent with unmasking cryptic localization sequences. Also, the paralogs exhibiting dependency were more likely to be heteromeric i.e. interacting with each other. Our study uncovers novel insights into the mechanisms of responsiveness among duplicated genes.

146A

Engineering Yeast with Catalytically Active Human Proteasome Core

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Yeast and humans share several thousand genes despite a billion years of evolutionary divergence. While many human orthologs are functionally replaceable in yeast, nearly half of the tested shared genes are not. These studies reveal a striking trend. Functional replaceability is not well-explained by sequence similarity between the human and yeast genes. Instead, it is a property of specific protein complexes and pathways referred to as "genetic modularity", such as some systems are near-entirely replaceable, whereas some modules are entirely non-replaceable. For example, while 7 of 7 yeast α proteasome core particle (CP) subunits are individually replaceable by their human counterparts, 5 of 7 contiguous subset of the β-ring subunits of CP are non-replaceable.
The modularity paradigm suggests testing if complete yeast and human systems are interchangeable. It also raises the hypothesis that an entirely humanized genetic module might replace the corresponding yeast system even when individual components are not humanizable due to specific genetic or protein-protein interactions that fail in the hybrid yeast/human system. We explore a novel strategy that does not need selection markers by using CRISPR-Cas9 and gene drives. The method enables Marker-less Enrichment and Recombination of Genetically Engineered loci (MERGE) in yeast. First, we establish the feasibility of MERGE by engineering most of the α proteasome core in yeast.
Next, we used robotic screening to assess thousands of non-replaceable human β2 proteasome gene variants for functional replaceability uncovering several functionally replaceable alleles. Notably, many suppressors reside on the diverged C-terminal tail loop interacting with the adjacent β3 subunit. To further prove the diverged role of the C-terminal tail loop in replaceability, swapping the full-length C-terminal from yeast to human β2 subunit allowed functional replaceability. Finally, we demonstrate that the catalytically active wildtype human β2 is functionally replaceable in a yeast strain harboring the adjoining human β3 (HsPSMB3) subunit. Our data reveal the role of species-specific protein-protein interactions in proteasome core governing functional replaceability. The yeast with catalytically active human proteasome subunits will serve as biological reagents to assay the impact of human genetic variation and gene-drug interaction on proteasome function while providing a synthetic platform to acquire novel therapeutics targeting the human proteasome.

147A

Fine-tuning phylogenomics for admixed *Saccharomyces cerevisiae*: problems and possible methodological solutions with short- and long-read sequencing

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The history of the yeast *S. cerevisiae* is deeply interwoven with that of human migrations, developments in agriculture and food technology, and trade since ancient times. Its use in fermentation and the consequent domestication process had made it one of the most important and most beneficial microorganisms. The species is a minor and probably transient member of the human microbiome, while it may also cause opportunistic infections.

Well over a thousand whole *S. cerevisiae* genomes have been sequenced internationally, making this yeast one of the best-known life forms in terms of intraspecific diversity. Yet, the species' fine-scale phylogeny and its domestication history are constantly being refined as new genomes are published from understudied sources. There is a consensus regarding the main clades of the species, but the high number of mosaic genomes with prevalent aneuploidy and polyploidy especially among domesticated lineages pose a significant challenge for phylogenomic methods, causing their placement to be unresolved.

In this work, we aimed to test and optimize phylogenomic methods for the evaluation of polyploid admixed yeasts. We generated various rearranged, introgressed, admixed, polyploid, aneuploid, and highly heterozygous genomes, and the combinations of these in silico. Then we simulated short-read sequencing of these. We also sequenced local baker's, wine, and probiotic yeast isolates with high coverage Illumina and ONT technology. With the simulated and real sequences, we compared two reference-based strategies, mapping to the species' S288C reference genome and mapping to multiple de novo assembled references. Subsequent allele calling and filtering practices were compared for these reference genome mappings. Maximum Likelihood-based and phylogenomic network methods were tested on the dataset, and whole-genome phylogenies were compared with results obtained for individual chromosomes, chromosomal regions and with consensus trees of individual gene trees.

Our results obtained for in silico and real genomes showed that in the case of admixed and highly heterozygous, polyploid genomes with structural variants, specifying chromosome-region copy number upon calling and the correct choice of representing phylogenomic relationships are especially important. We found SNP-network-based representation of smaller chromosome regions to be the most informative in understanding the origins of such yeasts that are common in domesticated environments.

148A

Pseudodiploidy enhances homology directed repair in *Saccharomyces cerevisiae*

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Cells have multiple repair pathways to repair a DNA double strand break. Foremost among them are homology-directed repair (HDR), a family of repair pathways that utilize homologous DNA as a repair template, and non-homologous end-joining (NHEJ), which acts without a repair template and can introduce indel mutations. Control of which repair pathway a cell uses increases the utility of genome editing technologies such as CRISPR. The gene *NEJ1* is a critical component of NHEJ in *Saccharomyces cerevisiae*. Diploid yeast express a1-alpha2, which naturally represses *NEJ1* and results in DNA repair via HDR. We have shown that mimicking this diploid state via expression of a1-alpha2 in haploid yeast results in DNA repair via HDR after a CRISPR-directed double stranded break, greatly increasing the rate of precise editing. RNA-seq data show repression of *NEJ1* in pseudodiploids and minimal perturbation of other cellular processes. To demonstrate the utility of pseudodiploidy in high-throughput yeast CRISPR experiments, we are using pseudodiploidy and CRISPR editing in a pool of mixed yeast to introduce unique barcodes linked to strain identity. This barcoded pool of diverse yeast can be used to easily determine growth differences between strains in various conditions in a highly replicated and controlled manner.

149A

Data Integration Through Allele Curation at SGD

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The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a model organism database with a goal of painting a complete picture of eukaryotic cellular processes by curating a comprehensive and diverse collection of data types for the budding yeast *Saccharomyces cerevisiae*. In addition to other data, we also have been capturing single mutant phenotypes as well as importing genetic and physical protein-protein interactions from BioGRID (thebiogrid.org) to help shed light on the role proteins play in cellular processes. While we have been collecting mutant types since we started annotating mutant phenotypes, we more recently decided to capture mutant alleles broadly and systematically. Users can now search for and find alleles associated with their genes of interest. In addition, pages dedicated to single alleles are now available and include information on allele type, molecular details of the mutation, alias names and associated references, along with all phenotype and interaction annotations where specific alleles were used. Alleles that share phenotype and/or genetic interaction annotations are graphically represented in the 'Shared Alleles' section. Users will be able explore more fully previously unlinked data types using our new allele curation model to gain additional insight into cellular processes and pathways. This work is supported by a grant from the NHGRI (U41 HG001315).

150A

The *Saccharomyces* Genome Database and the Gene Ontology: Best buds

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The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a comprehensive resource of curated molecular and genetic information on the genes and proteins of *Saccharomyces cerevisiae*. Since 2001, SGD has used the Gene Ontology (GO; geneontology.org) to annotate the functions of gene products in budding yeast. The GO comprises three sets of structured vocabularies, or "ontologies": the Molecular Function ontology describes activities of gene products; the Biological Process ontology places these molecular functions in a biological context; and the Cellular Component ontology indicates the subcellular localizations of gene products. Expert curators at SGD select appropriate GO terms to apply to gene products based on published scientific literature. At SGD, results from traditional experimental methods are the primary sources of evidence used to support GO annotations. Results from comparative sequence and genomic studies, as well as analyses of functional genomic and proteomic data, have provided valuable insights into the biological roles of gene products, and these data are also incorporated into SGD. SGD has several web interfaces and analysis tools that display and use these data. The Locus Summary page briefly lists each GO annotation; more GO information is on the Gene Ontology tab. Recently, SGD has begun to attach GO annotations to complexes, and these can now be found on the corresponding Complex pages. The GO Term Finder aids in discovery of potential gene similarities. SGD has developed an *S. cerevisiae*-specific GO Slim (or GO subset), a condensed version of the GO containing a subset of the terms. The GO Slim Mapper tool bins annotations of a group of genes to into broad categories. The Yeast GO Slim can be used in other ways including focusing on specific areas of GO or in searches and annotation processing. SGD prominently features the GO Slim terms as an overview of the function, process, and component GO annotations on both Locus and Complex Gene Ontology tabs. GO annotations are also incorporated into YeastMine (yeastmine.yeastgenome.org), SGD's multifaceted search and retrieval environment that provides access to diverse data types. These interfaces and tools are important as part of SGD's ongoing mission to facilitate research, education, and discovery using the Gene Ontology. This work is funded by the US National Institutes of Health: National Human Genome Research Institute (NHGRI [U41HG001315]) and National Institute of General Medical Sciences (NHGRI NIGMS [U41HG002273]).

151A

Integration of SGD regulatory and expression data into the GRNmap and GRNsight applications for modeling and visualizing small-to-medium gene regulatory networks

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A gene regulatory network (GRN) consists of genes, transcription factors, and the regulatory connections between them which govern the level of expression of mRNA and protein from genes. GRNmap is an open source MATLAB program that performs parameter estimation and forward simulation of a differential equations model of a GRN based on time course gene expression data. GRNsight is an open source web application for visualizing small-to-medium scale GRNs, especially models produced by GRNmap. GRNsight reads the Excel input and output workbooks from GRNmap and automatically displays the model data as a graph with colored nodes (expression data) and edges (estimated regulatory weights). A limitation for GRNmap has been the manual creation of input Excel workbooks which is time-consuming, error-prone, and dependent upon the user having their own network and expression data. To address this, we have implemented a backend PostgreSQL database for GRNsight, populated with five public gene expression datasets (Apweiler et al. 2012, GSE33098; Barreto et al. 2012, GSE24712; Dahlquist et al. 2018, GSE83656; Kitagawa et al. 2002, GSE9336; Thorsen et al. 2007, GSE6068) and regulatory network data from the *Saccharomyces* Genome Database (SGD). A user can now select genes to include in the GRN, and GRNsight will automatically layout the network using regulatory connections from SGD. The user can then select one of the time course gene expression datasets with which to color the nodes and export to a properly-formatted GRNmap input workbook. GRNmap can then be used to estimate the regulatory weights (activation vs. repression and magnitude of the relationship). A GRNmap executable is available for users who do not have access to a MATLAB license. Finally, the GRNmap output can be loaded back into GRNsight to visualize the results. Closing this loop for automating and validating the creation of GRNmap input workbooks speeds up the rate of research, enabling the comparison of models of different GRNs with the same expression data source or the same GRN with different expression data sources. GRNmap is available at http://kdahlquist.github.io/GRNmap/; GRNsight is available at http://dondi.github.io/GRNsight.

152A

A systematic approach to mapping bypass suppressors of essential genes and essential gene pairs in *S. cerevisiae*

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Genetic suppression occurs when a phenotype associated with mutation in one gene is rescued by a secondary, extragenic mutation. Dramatic examples of genetic suppression include bypass of the lethal phenotype associated with mutation in an essential gene, or of phenotypes associated with severe Mendelian disease mutations in humans. Previous efforts to map bypass suppressors of essential genes have involved screening for chemically induced or spontaneous mutations, followed by next generation sequencing which, although biologically informative, can be laborious and time consuming. Here, we present a systematic method for identifying bypass suppressors using the synthetic genetic array (SGA) method to introduce deletion mutations in essential query genes into yeast mutant arrays. Because the identity of the suppressor gene on the array is known, this method is rapid and enables exhaustive tests for all genes in the genome. We show that the pipeline recapitulates known suppressors (e.g. *isw2*Δ*)* and uncovers novel suppressors (e.g. *itc1*Δ*, sds3*Δ*, dep1*Δ*, sap30*Δ *and rxt2*Δ*)* the essential genes *ndd1*Δ. Furthermore, analysis of bypass suppression identified functions for poorly characterized genes, such as a role for *NAT2* in viral attenuation and mitochondrial function.

We also adapted the pipeline to identify bypass suppressors of synthetic lethal gene pairs – genes which when deleted alone are healthy but which cause lethality when combined together. We show that suppressors of different *BNI1*- synthetic lethal gene pairs are distinct and likely act through different mechanisms. For instance, deletion of *KIP2* or *BIK1*, which encode kinesin-related proteins, rescued the synthetic lethality of a *jnm1D bni1D double mutant* mutants while a hypomophic allele of *CDC37*, which encodes an Hsp90 co-chaperone, rescued a *slt2D bni1D* double mutant. These results suggest that systematic identification of bypass suppressors of synthetic lethal gene pairs will help illuminate the possible mechanistic underpinnings of synthetic lethality. In all, our pipeline offers a systematic and comprehensive approach to map suppressors of essential genes and synthetic lethal gene pairs, thereby illuminating the genetic network of a cell.

153A

Consequences of thiol oxidative stress on cytosolic proteostasis in yeast

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Neurodegenerative disease affects millions of Americans every year, through diagnoses such as Alzheimer's, Parkinson's, and Huntington's diseases. A core characteristic of these diseases is the presence of misfolded protein aggregates, often linked to decreased motor control and chronic injury to the brain. Although treatments exist, there are currently no cures for neurodegenerative pathologies. One factor linked as a precursor to the formation of these aggregates is damage sustained to proteins by oxidative stress, which can disrupt tertiary structure, causing misfolding. Cellular protein homeostasis (proteostasis) relies on the involvement of chaperone proteins, with the ubiquitous Hsp70 chaperone family playing a prominent role in prevention of aggregates and protection of misfolded protein precursors. Hsp70 activity has been shown to be affected by cysteine modification through oxidizing or thiol-modifying compounds. In the yeast cytosol, Hsp70 isoforms exist either as highly expressed, constitutive forms (Ssa1 and Ssa2) or as stress-inducible forms (Ssa3 and Ssa4). To investigate the biological consequences of cysteine modification on Ssa1, we attempted to generate a strain lacking all four SSA genes and expressing a mutant with aspartic acid substitution at C264 and C303 but were unable to do so, demonstrating that oxidation or modification of these residues may be unsustainable for cellular growth. Cells lacking *SSA1* and *SSA2* and expressing the same allele were viable but slow-growing, and this strain was utilized to probe the impacts of Ssa1 cysteine modification on Hsp70 biological roles including repression of Hsf1, protein biogenesis and folding, and chaperone-mediated degradation of misfolded proteins. Using an Hsf1-controlled expression reporter, the C264D/C303D *SSA1* mutant was shown to constitutively activate Hsf1 and elevate expression of downstream stress-related

genes. Luciferase activity assays revealed a reduced capacity to correctly fold the nascent reporter protein FFL-GFP, and to refold FFL-GFP after heat-induced misfolding. Ongoing cycloheximide chase experiments suggest significant deficiency in Ssa1-dependent degradation of chronically misfolded proteins. Taken together, these experiments demonstrate that modeling cysteine modification in the constitutive cytosolic chaperone Ssa1 results in a significant impact on several of its roles in proteostasis. Future experiments will examine the ability of modified Ssa1 to bind, hydrolyze, and release nucleotide *in vitro* to assess the potential biochemical causes of the *in vivo* deficiencies. Understanding how oxidation affects not only proteins, but also the protective systems that prevent and manage insults to proteostasis increases our ability to comprehend the complex disease state surrounding neurodegenerative disorders and more effectively treat them.

154A

Integrative analysis of genomic loci linked to gene expression and growth reveals causal genes and mechanisms underlying complex traits

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Considerable effort is directed at understanding the genes & mechanisms that underlie variation in quantitative traits. A common strategy integrates trait loci detected by genome-wide association studies with expression quantitative trait loci (eQTLs), genomic regions harboring DNA variants that alter gene expression. Recent studies in humans have shown that known eQTLs account for a surprisingly small fraction of complex trait heritability. However, human eQTL datasets have low statistical power, limiting their utility for understanding causal mechanisms.

Saccharomyces cerevisiae is a key species for understanding complex traits. Mapping in 1000 recombinant progeny of the BY & RM strain cross has yielded comprehensive sets of eQTLs & growth QTLs (gQTLs) that account for most of the heritability in gene expression and growth in 46 environmental conditions. Here, we integrate these highly powered eQTL and gQTL data towards identifying causal genes and mechanisms underlying complex traits.

The expression of hundreds of genes showed statistically significant correlations with growth in at least one condition. There was significant overlap between the eQTLs of these genes and the respective gQTLs, suggesting a genetic basis for the observed phenotypic correlations. We performed extensive colocalization analyses to evaluate if gQTLs are likely caused by the same causal DNA variants as the eQTLs they overlap. Of 2,361 examined gQTL/eQTL pairs, 1,669 (69%) were consistent with shared causal variants. At 54% of these pleiotropic loci, mediation analysis suggested that these loci drive variation in growth by modulating the given gene's expression. In all, we detected 325 causal genes across 25 traits, providing leads into the mechanistic basis of complex trait variation.

Our yeast data include thousands of *trans*-eQTLs, which influence the expression of distant genes and are the main source of regulatory genetic variation. The many yeast *trans*-eQTLs enabled discovery of cases in which several gQTLs for the same trait all act as independent *trans*-eQTLs that modulate the expression of the same gene. For example, 6/15 gQTLs for growth in SDS showed significant mediation by *PIL1*, suggesting that the precise expression of *PIL1* (which plays roles in the plasma membrane) is an important factor shaping the ability of yeast to grow in SDS.

Collectively, our results show how comprehensive yeast QTL data can nominate novel causal genes and mechanisms underlying complex traits.

155B

Contaminated medicines: The role of arginine biosynthesis in N-nitrosamine toxicity

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The recall of several millions of medications since 2018 are due to contaminations by N-nitrosamines, including N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). Initially uncovered as contaminants in Losartan/Valsartan, N-nitrosamines have now been detected in Metformin, Ranitidine, and Rifampin/Rifapentine, among others. As recently as April 2022, more batches of these drugs are still being recalled, while other drug types (e.g Quinapril) are also being found to contain N-nitrosamines. These reactive molecules comprise a class of toxic contaminants that have been found to be genotoxic and carcinogenic in at least 40 experimental animals, likely through formation of DNA adducts. The pervasiveness of these toxic compounds raises great concerns, especially because the long-term effects on patients are largely unknown. For instance, little is known about their interactions with genes or proteins, or even pathways that could mediate their toxic effect. We applied yeast chemical genomics assays to identify genes that might mediate N-nitrosamine toxicity. Using competitive parallel deletion mutant analysis and next generation sequencing, we showed that strains deleted for arginine biosynthetic genes, such as ARG3 (ornithine carbamoyltransferase), are hypersensitive to NDMA and NDEA. To understand why these genes were particularly affected by these contaminants, we tested their metabolic intermediates and found that they also caused hypersensitivity to deletion mutants of the arginine biosynthesis. Additional studies showed that the effect on arginine biosynthetic pathway could be in part, through the formation of ammonium during their biotransformation. We further showed that this effect on the arginine biosynthetic pathway is independent of arginine transport, and that antioxidants could not relieve the effect. Finally, we showed that overexpression of Arg3p and other arginine biosynthetic proteins had a profound protective effect against N-nitrosamine toxicity in cells. Our work provides a rich resource for further work on the toxicity mechanism of N-nitrosamines.

156B

Analysis of ~10,000 CRISPR interference perturbations in a yeast cross

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Genetic perturbations can show different phenotypic effects across individuals due to epistasis with segregating loci. To achieve a broader understanding of the extent and genetic basis of these background effects, we developed a yeast cross amenable to high-throughput integration and efficient phenotyping of CRISPR interference libraries. In our initial experiment, we examined ~200 segregants, each containing a library of ~10,000 gRNAs targeting primarily essential genes. The relative fitnesses of all segregant-gRNA combinations were measured in a common pool using a double-barcode sequencing strategy, with one barcode denoting a segregant genotype and a second barcode denoting a gRNA. Preliminary results indicate a relationship between the effect of a gRNA in a segregant and the fitness of that segregant, with lower-fitness genotypes often exhibiting both more gRNAs with effects and a larger magnitude of detected effects. While analysis is ongoing, we expect these data will enable a broad, systems-level understanding of how genetic differences among individuals cause background effects.

157B

Quantitative trait gene discovery by genome-wide reciprocal hemizygote scanning

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Genetic variation among individuals influences many important traits, including common human disease. Quantitative trait locus (QTL) mapping in model organisms has revealed that most quantitative traits are affected by multiple QTLs throughout the genome. However, identifying the causal genes within QTLs (quantitative trait genes; QTGs) remains challenging because most QTLs are wide and can contain dozens of genes. Experimental fine-mapping approaches typically test causality one gene at a time. This process is both laborious and potentially biased towards genes previously shown to affect the trait. An unbiased, systematic approach for direct QTG identification is advantageous.

To systematically identify QTGs for a model complex trait, we applied genome-wide reciprocal hemizygote (RH) scanning to the growth of *Saccharomyces cerevisiae* in culture. In an RH test, two genetically different strains are crossed to form a diploid hybrid. Knocking out one allele of a given gene creates a “hemizygous” genotype. This strain is compared to the “reciprocal” strain, in which the corresponding allele on the homologous chromosome is knocked out. A phenotypic difference between the reciprocal strains reveals the gene to be a causal QTG.

To apply the RH test genome-wide, we follow recent advances in interspecies hybrids by using the *piggyBac* transposon to mutagenize a hybrid between two genetically different *S. cerevisiae* strains to yield a large reciprocal hemizygote pool. We used Illumina sequencing of transposon insertion sites to count insertions at each open reading frame (ORF) in the pool. Out of the 4,784 ORFs that carry DNA variants between the two parental strains of the hybrid, 4,440 contained at least one insertion, with 4,260 ORFs containing at least one insertion in both alleles. This comprises ~65% of all yeast ORFs.

We grew replicates of the hemizygote pool in nutrient-rich media for approximately 70 cell divisions and tracked insertion frequencies at multiple timepoints. Genes with a significant allelic difference in change in insertion frequency over time are considered QTGs. Using a custom computational pipeline and linear modeling, we identified 265 genes with at least a nominally significant ($p < 0.05$) allelic effect on growth. We are currently experimentally validating the most significant QTGs. Revealing QTGs aids in understanding how genetic variation affects important cellular traits such as growth and can be readily applied to other phenotypes.

158B

Genetic-Interaction Screens Identify Functional Redundancy and Regulators of Transcription Factors in Fission Yeast

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Transcription factors are central in linking signalling pathways to gene expression programs in response to various environmental and physiological conditions. Most transcription factor deletion strains in *Schizosaccharomyces pombe* are viable with no obvious phenotypes in rich medium, which may indicate functional redundancy. In contrast, overexpression of most transcription factor genes results in reduced fitness. We performed systematic synthetic sick/lethality (SL) and synthetic dosage lethality (SDL) screens in *S. pombe* to determine the degree of functional redundancy between transcription factor pairs and identify potential regulators of transcription factors, respectively. Intercrossing 38 query and 92 array transcription factor deletion strains by synthetic genetic array (SGA) generated 2714 double mutants with a SL interaction frequency of ~1.77%. Phenotypic analysis of double mutant strains revealed potential cell cycle roles for several poorly characterized transcription factors including SPBC56F2.05, SPCC320.03 and SPAC3C7.04. In addition, a miniarray of 279 strains containing deletion of genes that encoded primarily posttranslational-modifying enzymes was mated to 14 transcription factor overexpression strains by SGA and subsequently assayed for SDL interactions. The frequency of SDL interactions obtained in these screens was ~4.99% and consisted of known and novel regulators often implicated in similar cellular processes as the transcription factor. We discovered that the ubiquitin ligase Ubr1 and E2/E3-interacting protein Mub1 act to degrade the glucose-responsive transcriptional repressor Scr1. Loss of *ubr1*$^+$ or *mub1*$^+$ resulted in induced activity of Scr1 by increasing intracellular protein expression and enhancing its

novel function in repression of flocculation. Our study demonstrates that SL and SDL screens can be effective in uncovering novel functions and upstream regulators of *S. pombe* transcription factors.

159B

Systematic exploration of Complex HaploInsufficiency (CHI) in *Saccharomyces cerevisiae*

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Genome-wide Association (GWA) studies have identified many disease-associated variants that explain a portion of the heritability of genetic diseases; however, a large proportion of heritability remains unexplained, an effect known as the “missing heritability problem”. A component of missing heritability can be attributed to genetic interactions (GIs), defined as an observed phenotype in a double mutant that cannot be explained by the additive effect of both single mutants. Systematic analysis of all possible double mutant combinations in haploid *Saccharomyces cerevisiae* revealed ~1 million GIs, providing a wealth of information about the interconnectivity between core processes and biological pathways of a cell. While highly informative, identifying GIs in a haploid cell does not account for the effects of diploidy or heterozygosity, essential features of mammalian genomes. Complex HaploInsufficiency (CHI) is a GI that occurs because of heterozygosity at two more loci in the genome and is a diploid-specific GI that remains unexplored on a global scale. To systematically explore CHI genetic interactions, we developed an automated screening pipeline to generate diploid mutants that are heterozygous for temperature sensitive (ts) alleles of essential genes, which are functional at permissive temperature but loss-of-function at high temperature, at two loci in the genome. Using colony size as a quantitative metric of fitness, we screened all possible essential x essential gene pairs (~800,000) and identified ~1100 negative and ~450 positive CHI pairs, which are enriched for, but largely non-overlapping with haploid GIs. CHI interactions are rare, involving ~0.2% of all possible pairs compared to 10% of pairs having a GI in haploid cells. Nonetheless, genes that show CHI interactions have several clear features: [1] less fit single heterozygotes have more interactions compared to more fit heterozygotes; [2] CHI gene pairs tend to belong to same broad functional class (but not specific bioprocesses); and [3] the majority (87%) of CHI gene pairs do not show GIs in haploids. CHI hubs are most prominently enriched for “ribosome/translation”, “protein turnover/proteasome” and “polarity/morphogenesis”, suggesting that diploid genomes are less able to buffer perturbations of protein quality control and cell structure than other processes. Nevertheless, the relative scarcity of CHI pairs suggests that an additional copy of each gene confers a strong resistance to loss-of-fitness from the combinatorial effects of mutations in the genome.

160B

Detection and quantification of genetic background effects using genome-wide, double barcoded, CRISPRi perturbations in a focused yeast cross

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The phenotypic effects of mutations often depend on the genetic backgrounds in which they occur. Our goal is to move towards a fundamental understanding of the genetic and molecular mechanisms producing these background effects, using the budding yeast model system. Here, we will leverage a double barcoding scheme to precisely measure the fitness effects of genome-wide CRISPR interference perturbations in a panel of 14 genetically diverse haploid and diploid S. cerevisiae strains. Each strain will be integrated with 8,886 and 10,795 CRISPR guides respectively targeting every reported essential (n=1,642) and nonessential gene (n=3,832). Relative fitnesses of all strain-guide combinations will be measured in a pooled competition by quantifying each double barcode through sequencing. We will analyze ≥3 barcode replicates per strain and ≥20 barcode replicates per guide. This high internal replication will provide the statistical power to detect guides that have different phenotypic effects across strains. With these data, we will determine the prevalence, extent, and character of genetic background effects across genetic perturbations. Additionally, we will explore the roles that ploidy and heterozygosity have on background effects.

161B

Systematic analysis of temperature-sensitive alleles of essential genes uncovers new regulators of filamentous growth in yeast

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Through the process of differentiation, cells can specialize into different types with distinctive shapes and specific functions. Many fungal species undergo filamentous growth, where cells differentiate to pseudohyphal and hyphal cell types in response to environmental stresses, like nutrient starvation. Fungal species also form biofilms or mats, where communities of cells adhere to each other and to surfaces through the regulated expression of genes responsible for cell adhesion. In opportunistic fungal pathogens, like *Candida albicans*, these responses contribute to pathogenicity. The budding yeast, *Saccharomyces cerevisiae,* also undergoes filamentous growth and forms biofilms/mats. Genetic screens and nonessential gene-deletion collections have identified many regulators of these processes. However, one group of genes that have yet to be systematically analyzed for their role in filamentous growth and biofilm/mat formation are genes with essential

functions. We designed an approach to transfer a collection of temperature-sensitive (ts) alleles of essential genes into a strain background (∑1278b) that undergoes filamentous growth. To date, 135 ts alleles representing 44% of essential processes have been introduced into the filamentous background. Ts strains were examined at semi-permissive temperatures for phenotypes in invasive growth, pseudohyphal growth, and biofilm/mat formation. New roles were uncovered for essential genes that function in processes including organization of the actin cytoskeleton, GPI-anchor biosynthesis, splicing, kinetochore assembly, and chromosome segregation that impact filamentous growth. We also uncovered a key role for the Wiskott-Aldrich Syndrome protein (WASp) homolog, Las17p, in regulating the Cdc42p-dependent MAP kinase pathway (fMAPK) that controls filamentous growth. Unexpectedly, Las17p did not impact another Cdc42p-dependent MAPK pathway that controls mating and shares components with the fMAPK pathway. The tetra-span sensor of the fMAPK pathway, Sho1p, was present at reduced levels and was mis-localized in the *las17-13* mutant, which might account for the specific role of Las17p in regulating the fMAPK pathway. This type of approach may improve our understanding of the roles that highly conserved and ubiquitously present essential genes play in cell differentiation. Moreover, the collection may also provide a resource for comparing essential gene function among individuals of the same species.

162B

Investigating the genetic and metabolic changes in response to DNA damage stress in telomerase-mutant yeast Jennifer Gallagher, Taizina Momtareen West Virginia University

Cancer cells can elongate telomeres using the ALT pathway of DNA recombination when telomerase is not expressed. Some telomerase mutants of S. cerevisiae also proliferate indefinitely using ALT-like processes, making these yeasts (aka survivors) a great model to study such immortal cells. The goal of this study is to develop a deeper understanding of how survivor cells, specifically the type II survivors, function in genomic and metabolic levels. The change in the growth rate and metabolite levels throughout the lifespan of a telomerase mutant cell will give insight into their stress response mechanisms. Examining their recombination-based telomere elongation will help determine which genes play key roles in their maintenance. For example, type II survivors are not generated in the absence of the DNA helicase encoding gene SGS1. To identify genes that can functionally replace SGS1, we have conducted an overexpression genetic screen where the entire yeast genome was transformed into sgs1 mutants. These cells were then grown on the DNA damaging agent hydroxyurea to identify suppressors and expected suppressors were found such as genes encoding nucleotide excision repair (RAD3, RAD4, RAD10), histone demethylase (RPH1), and RNA helicases (UPF1, IRC5). . Novel suppressors will prioritized in future work. This screen will discover genes that can potentially generate type II survivors in the absence of SGS1. These metabolic and overexpression studies will give us insight on how cells can be functional under extreme replicative stress.

163B

Chromosome Substitution for Characterizing Epistasis Cassandra Buzby, Mark Siegal New York University

Complex traits are the products of multiple genes and environmental factors, yet how these influences interact largely remain a mystery. The contribution of genetic interactions (epistasis) to natural trait variation is particularly challenging to estimate experimentally, and current approaches for detecting epistasis are often underpowered. Powerful mapping approaches such as bulk segregant analysis, wherein individuals with extreme phenotypes are pooled for genotyping, obscure epistasis by averaging over genotype combinations. To accurately characterize and quantify epistasis within natural trait variation, we have engineered Saccharomyces cerevisiae strains to enable crosses in which one parents' chromosome is fixed while the rest of the chromosomes segregate. Specifically, we placed an inducible promoter upstream of the targeted chromosome's centromere, which upon induction destabilizes the centromere via transcription and results in loss of the chromosome. Bulk segregant analysis then allows us to compare quantitative trait loci whose effects depend on alleles on the fixed chromosome for phenotypes such as chemical resistance. QTL identified by bulk segregant analysis in the context of one parents' fixed chromosome compared to the other indicate a genetic interaction with that chromosome. Using this method, we can thus identify interacting loci with high statistical power.

In a cross of a strain derived from a wine barrel ("wine") and a strain derived from an oak tree ("oak"), we obtained large pools of segregating progeny fixed for chromosome I from oak or wine. From each pool, we selected bulks grown in either YPD media or media containing CuSO4 to map effects that do or do not depend on the parent of origin of chromosome I. Interestingly, we found no epistasis with chromosome I, indicating that this small chromosome does not interact with other loci for this trait, but we did detect additive effects with high power. We aim to next test different traits for epistasis with chromosome I, and other chromosomes for epistasis for CuSO4 resistance.

164B

Characterization of lysine transport at the yeast vacuolar membrane and study of its physiological role Evi Zaremba[1], Anna Dodinval[1], Fabienne Vierendeels[2], Elisabeth Bodo[2], Bruno André[1], Melody Cools[21] Université Libre de Bruxelles, [2]Labiris

The vacuole of the yeast *Saccharomyces cerevisiae* serves as a storage compartment for a large array of metabolites that cells can utilize to survive environmental changes and nutrient shortages. Particularly, more than 90% of the cationic amino acid pools of the cell are sequestered in the vacuole[1]. The transporters involved in arginine import and export across the vacuolar membrane have recently been identified as Vsb1 and Ypq2 respectively[2].

How lysine is compartmentalized and mobilized according to environmental cues, however, remains poorly understood. A paralogue of Ypq2, the Ypq1 transporter, has been shown to import lysine into isolated vacuolar vesicles[3]. Additionally, *vsb1*Δ mutant cells exhibit a lower total lysine pool than *w-t* cells, suggesting that Vsb1 can transport lysine as well as arginine[4].

In this work, we investigate the role of these proteins in lysine transport at the vacuolar membrane using a combination of amino acid pool measurements, growth tests and uptake assays in whole cells and isolated intact vacuoles. First, we find that Vsb1 is essential for lysine accumulation into the cell and the vacuole. Second, we observe that Ypq1 is implicated in the mobilization of vacuolar lysine. All in all, our results suggest that lysine is transported into the vacuole by Vsb1 and is exported to the cytosol by Ypq1 under lysine scarcity. Further investigations will be required to understand how these transporters are regulated.

During our study, we observed that the cellular pools of all amino acids decrease under nitrogen starvation except for the lysine pool which surprisingly increases. We hypothesize that this increase could be due to *de novo* lysine biosynthesis, endocytosis of plasma membrane proteins and/or macroautophagy[5]. We thus first quantify the expression of several genes involved in these processes and confirm that lysine biosynthesis and macroautophagy are indeed induced after a shift to a nitrogen free medium. Additionally, we measure the lysine pools of mutants hindered for biosynthesis (*lys14Δ*), multivesicular body (MVB) protein sorting (*vps4Δ*) and macroautophagy (*atg8Δ*). Preliminary results show that lysine accumulation under nitrogen starvation is heavily impaired in biosynthesis and autophagy mutants suggesting that both processes contribute to this increase in lysine levels. Studies are now in progress to understand the physiological role of vacuolar lysine in these conditions.

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165B

A screen for histone mutations that affect quiescence and chronological lifespan in *S. cerevisiae*.

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Quiescence is a distinct cell cycle phase, termed G0, in which growth, transcriptional and translational activity, and replication are halted. Yeast cells enter G0 following glucose exhaustion but remain viable for an extended period and can re-enter the cell cycle when returned to glucose rich medium. Quiescence is a feature of all organisms and is essential for the maintenance of stem cells and tissue renewal. Quiescence is also related to chronological lifespan (CLS) - or the ability of post-mitotic quiescent cells to survive over time - and thus contributes to the longevity of yeast populations. However, important questions remain to be answered regarding the mechanisms that control entry into quiescence, the maintenance of quiescence or chronological lifespan, and the re-entry of quiescent cells into the cell cycle.

During the formation of quiescent yeast cells histone acetylation is lost and chromatin becomes highly condensed. This unique chromatin landscape plays a key role in supporting quiescence-specific transcriptional repression and has been linked to the formation and maintenance of quiescent cells. To ask if other chromatin features might also alter quiescence or CLS, we conducted a comprehensive screen of H3 and H4 histone mutants. We identified a number of mutants that show altered quiescence and have characterized their chromatin phenotypes. Initial analysis showed that none of these mutants retain histone acetylation, while several of the mutants have altered chromatin condensation. Additionally, the screen for H3 and H4 mutants with altered chronological lifespan showed that CLS is highly correlated with quiescent cell formation.

166B

Genetic Network Rewiring Between Distantly Related Yeast Species

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Synthetic lethality represents an extreme example of a genetic interaction that occurs when a combination of mutations in different genes results in lethality, which would not be expected from the combined effects of individual viable single mutants. The extent of genetic interaction network conservation does not directly translate to the extent of genome sequence conservation between species. Two distantly related yeast species, the budding yeast, *Saccharomyces cerevisiae*, and fission yeast, *Schizosaccharomyces pombe*, diverged 500 million to 1 billion years ago and previous studies showed that despite displaying 75% genome conservation, there is only 29% genetic interaction network conservation between these two yeast species. To understand the full extent of genetic interaction conservation, this project aims to investigate cases of genetic network rewiring by studying the genetic interactions that underlie the change inessentiality status of single and double mutants between *S. cerevisiae* and *S. pombe*. First, PomBase was used to curate essential genes and BioGRID was used to curate synthetic lethal double mutants in *S. pombe*. Second, *S. pombe* and *S. cerevisiae* orthologs were mapped using PomBase. Third, our previously published datasets were used to identify nonessential single and double mutants of orthologous genes in *S. cerevisiae*. Then, instances of rewiring were revealed by identifying synthetic lethal digenic interactions that rewire nonessential single mutants to synthetic lethal double mutants in *S. cerevisiae*. In the future, the rewiring of nonessential *S. cerevisiae* double mutants to synthetic lethal triple mutants will be identified using a high throughput screening method, trigenic Synthetic Genetic Array (τ-SGA). Preliminary analysis shows that rewired *S. cerevisiae* genes are involved in DNA repair and replication, metabolism, and vesicle trafficking biological processes. The genes that are rewiring them representing digenic synthetic lethal interactions are co-annotated to the same biological processes. This work provides insight into the genotype-to-phenotype relationships and the principles of conservation of genetic interaction networks.

167V

Production of soybean ferritin variants with cadmium-binding affinity by *in vivo* cloning and mutagenesis in *Saccharomyces cerevisiae*

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Ferritin is a multimeric protein that stores thousands of ferric ions in plants, animals, and numerous microorganisms. One important exception is *Saccharomyces cerevisiae*, which has other means to store iron but, remarkably, can produce high levels of functional ferritins from different sources. For example, the expression of the soybean ferritin in yeast causes iron accumulation and increased resistance to the metal[1]. The selectivity toward iron is based on the ferroxidase activity within the protein structure, which allows the oxidation of the ferrous ions and thus, their eventual efficient storage. Additionally, ferritins also exhibit affinities to other metal ions such as cadmium, and important examples are those from pea and horse[2,3]. A comparison of the amino acid sequences of these ferritins allowed the identification of a region that might be responsible for the different specificities. To study this possibility, hybrid soy-pea ferritin variants were produced in yeast cells, which interestingly, exhibited increased accumulation and resistance to cadmium. Thus, to further increase the affinity for this metal, the same region was subjected to random mutagenesis. The *in vivo* cloning approach allowed the efficient construction of a plasmid library and the immediate expression of the variants in *S. cerevisiae*. The generated plasmid library was screened to identify the ferritin variants that conferred increased resistance to Cd^{2+}. As a result, one, named T14, was identified that allowed the growth of the yeast transformants in media containing up to 80µM $CdCl_2$. Additionally, to reduce the affinity of the variants towards iron, the amino acid residues responsible for the ferroxidase activity were also mutated. All variants were then characterized to compare their affinities toward cadmium and iron, and their capacities to remove Cd^{2+} from the media. Lastly, since ferritin is a thermostable protein, protein extracts were treated at 75°C for 15 min, and the supernatants were subjected to analysis by SDS-PAGE and cadmium content. These assays demonstrated that the ferritin variants had the expected sizes and further, that the T14 variant without ferroxidase activity had the highest Cd^{2+} content. Accordingly, up to 80% cadmium removal was achieved using these variants, suggesting the possibility of their potential application in bioremediation. In conclusion, these results demonstrate the feasibility of redesigning ferritin functions using an *in vivo cloning* strategy in yeast.

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168V

Global analysis of genetic suppression of partial loss-of-function alleles

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Genetic suppression occurs when the deleterious phenotype of a particular mutation can be rescued by another genomic alteration. Systematic analyses of suppression interactions in yeast and other model organisms have provided important insights into the general properties of genetic suppression and uncovered functional relationships between genes and pathways. However, although complete absence of a gene is rare among natural or disease-causing genetic variants, these studies predominantly relied on deletion alleles to identify suppression interactions. Here, we took advantage of a collection of temperature sensitive (TS) alleles of essential yeast genes to uncover and understand principles of suppression of partial loss-of-function alleles. We isolated a total of 2467 strains with mutations that suppressed the TS phenotypes of their respective parents, encompassing 995 TS alleles of 651 essential genes. We could isolate suppressors of temperature sensitivity for 89% of the essential genes in our collection. In contrast, only 17% of the deletion alleles of these genes could be suppressed in a previous study, indicating that distinct strategies are employed for complete and partial loss-of-function suppression. Whole-genome sequencing identified 1212 candidate suppressor mutations that frequently highlighted new connections between functionally related genes. Another ~9% of the suppressors involved general suppression mechanisms that affect the expression or stability of the TS mutant mRNA or protein and ~21% of the suppressor mutations occurred in the TS allele. In addition, sequencing of the parent TS alleles revealed the mutations underlying TS phenotypes. Our initial analysis of these TS mutations shows that they often affect protein stability and occur at highly conserved sequences or on interaction interfaces. Finally, we explored the genomic positions of the TS mutations to pinpoint suppression interactions that are specific to the mutation of a particular gene domain. This work is generating the most extensive global suppression network for a eukaryotic cell, identifying novel functional connections between genes, and improving our understanding of how mutations can interact to produce unexpected phenotypes.

169A

Yeast Nucleolin Nsr1 Impedes Replication and Elevates Genome Instability at an Actively Transcribed Guanine-Rich G4 DNA-Forming Sequence

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A significant increase in genome instability is associated with the conformational shift of a guanine-run-containing DNA strand into the four-stranded G-quadruplex (G4) DNA. The mechanism underlying the recombination and genome rearrangements following the formation of G4 DNA *in vivo* has been difficult to elucidate but has become better clarified by the identification and functional characterization of several key G4 DNA-binding proteins. Mammalian nucleolin (NCL) is a highly specific G4 DNA-binding protein with a well-defined role in the transcriptional regulation of genes with associated G4 DNA-forming sequence motifs at their promoters. The consequence of the *in vivo* interaction between G4 DNA and nucleolin in respect to the genome instability has not been previously investigated. We show here that the yeast nucleolin Nsr1 is enriched at a G4 DNA-forming sequence *in vivo* and is a major factor in inducing the genome instability associated with the cotranscriptionally formed G4 DNA in the yeast genome. We also show that Nsr1 results in impeding replication past such a G4 DNA-forming sequence. The G4-associated genome instability and the G4 DNA-binding *in vivo* require the arginine-glycine-glycine (RGG) repeats located at the C-terminus of the Nsr1 protein. Nsr1 with the deletion of RGG domain supports normal cell growth and is sufficient for its pre-rRNA processing function. However, the truncation of the RGG domain of Nsr1 significantly weakens its interaction with G4 DNA *in vivo* and restores unhindered replication, overall resulting in a sharp reduction in the genome instability associated with a guanine-rich G4 DNA-forming sequence. Our data suggest that the interaction between Nsr1 with the intact RGG repeats and G4 DNA impairs genome stability by precluding the access of G4-resolving proteins and impeding replication.

170A

Mechanisms involved in differential telomere length homeostasis.

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Telomeric DNA is composed of short tandem repeats of TG-rich DNA sequences that end in a single-strand extension of the 3′-end. Repeat length is an important regulator of cell proliferation potential. According to a strong tenet in the field, all telomeres in a cell are of similar or standard length; for example, in budding yeast, all telomeres are thought to comprise 300±75 bp of telomeric repeats. From yeast to humans, there is some evidence that subtelomeric chromatin and genomic loci located just inside of telomeric repeats can affect telomere length homeostasis in *cis*. However, nothing is known about how this regulation comes about. Counter to this established tenet, we recently discovered that in *S. cerevisiae*, the telomere from the left arm of chromosome III (TEL03L) is almost twice as long as the so-called 'standard' telomere length. Remarkably, deletions of SIR3 or SIR4 abolish this over elongated phenotype on TEL03L and the telomere now conforms to the 'standard' length. We hypothesize that a particular region in the subtelomere of TEL03L is responsible for this intriguing behavior. I will present results with altered TEL03Ls in which various subtelomeric areas are deleted or imported from other telomeres. The goal of this project is to assess whether and how subtelomeric regions are responsible for deviating telomere length phenotypes, such as that detected at TEL03L, and characterize the mechanisms involved in it. Moreover, extending this idea leads us to propose that each telomere may have its own length equilibrium that is dependent on cis- and trans-acting factors.

171A

Phosphorylation-mediated Ccp1-Ndc80 switch at the N-terminus of CENP-T regulates kinetochore assembly in fission yeast

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Kinetochores, a protein complex assembled on centromeres, mediate chromosome segregation. In most eukaryotes, centromeres are epigenetically specified by the histone H3 variant CENP-A. CENP-T, an inner kinetochore protein, serves as a platform for the assembly of the outer kinetochore Ndc80 complex during mitosis. How CENP-T is regulated through the cell cycle remains unclear. Ccp1 (*c*ounteracter of *C*ENP-A loading *p*rotein 1) in fission yeast associates with centromeres during interphase, but delocalizes from centromeres during mitosis. Here we demonstrated that Ccp1 directly interacts with CENP-T. CENP-T is important for the association of Ccp1 with centromeres, whereas CENP-T centromeric localization depends on Mis16, a homolog of human RbAp48/46. We identified a conserved Ccp1 interaction motif (CIM) at the N-terminus of CENP-T, which is adjacent to the Ndc80 receptor motif. The CIM domain is required for Ccp1 centromeric localization. The CIM domain-deleted CENP-T mutant phenocopie *ccp1*D. We further found that the CIM domain can be phosphorylated by CDK1 (Cyclin-Dependent Kinase 1). Phosphorylation of CIM weakens its interaction with Ccp1. Consistent with this, Ccp1 dissociates from centromeres through all stages of the cell cycle in the phosphomimetic mutant of the CIM domain, whereas in the phospho-null mutant of the domain, Ccp1 associates with centromeres during mitosis. We further show that the phospho-null mutant disrupts the positioning of the Ndc80 complex during mitosis, resulting in chromosome missegregation. Our results suggest that CDK1-mediated phosphorylation of the motif at the onset of mitosis promotes the switch of Ccp1 to Ndc80 at the N-terminus of CENP-T, resulting in dissociation of Ccp1 and proper assembly of Ndc80. At the end of mitosis, the domain is dephosphorylated, leading to reassociation of Ccp1 with CENP-T. Our results reveal a previously unrecognized mechanism underlying kinetochore assembly through the cell cycle.

172A

Deletion of telomeres via genetic engineering of circular chromosomes in *S. cerevisiae*

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Chromosome structure varies from a single circular chromosome in prokaryotes to multiple linear chromosomes in eukaryotes. Linear chromosomes differ in that they possess specialized ends, called telomeres, that play important roles in protecting the ends from degradation. Furthermore, telomeres cannot be fully copied during DNA replication without the enzyme complex telomerase. Since telomerase is not expressed in all cells, telomeres shorten with aging. On the other hand, overexpression of telomerase is observed in >85% of human cancers. Given that telomeres create this end-replication problem, our lab is interested in ultimately understanding why linear chromosomes evolved. To address this broad question, we are taking a novel genetic approach to systematically convert each of the linear chromosomes into circularized versions in *Saccharomyces cerevisiae*. Our approach involves building two DNA cassettes with selectable marker elements that are integrated into the left and right arms of each chromosome in a haploid yeast strain. The cassettes are designed so that recombination between the two regions will join the ends and reconstitute a functional marker gene, allowing genetic selection of circularized chromosomes. To date, we have isolated a circularized version of Ch. IV and Ch. VIII. We have verified recombination between the cassette via PCR across the newly formed junction. We are currently analyzing growth rates and colony morphology, cell size and morphology, mating and sporulation, and gene expression. Initial tests have failed to show any differences between the circular and linear versions of these two chromosomes. Interestingly, four other chromosomes have failed to yield viable circularized versions. We are currently trying to determine whether these are truly inviable as circles or whether the location of the junction needs to be optimized. However, out data demonstrate that our approach can generate viable yeast with individual chromosomes circularized. A longer-term goal of the lab is to use this approach to circularize the remaining chromosomes and explore how linear chromosomes evolved.

173A

Centromeric sequence variation is widespread and affects cellular fitness

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Centromeres, chromosomal sites of kinetochore attachment, have a highly conserved function which is essential for chromosome segregation. Despite functional conservation, the sequence of centromeric DNA is highly variable both between and within populations, including humans. Precisely altering these sequences has been difficult owing to the highly repetitive nature of centromeric sequences. Due to this intractability, no studies have examined the functional consequences of centromere variation in true genetic isolation. We addressed this challenge using *Schizosaccharomyces pombe* as a model. *S. pombe* centromeres are composed of core sequences, where kinetochores assemble, and flanking heterochromatin-nucleating repeat arrays. We analyzed the centromeric sequences from a wide variety of *S. pombe* natural isolates and characterized variation at the level of sequence and copy number. We found that the absolute size of the centromeres varies dramatically (35-180 kb), with most of the variation stemming from a difference in the copy number of the outermost repeats at centromere 3 (CenIII). To test the consequences of this variation, we constructed genetically identical *S. pombe* lab isolate strains containing either the endogenous “medium” CenIII allele (102 kb) or a “small” CenIII allele (35 kb) from a different natural isolate. We found that the medium CenIII allele had a significant fitness advantage when competed against the small CenIII allele in mitotically growing co-culture. This finding demonstrates that changes in centromeric sequences impact fitness. Furthermore, we speculate that *S. pombe* can serve as a valuable model to understand the functional significance of extant centromere variation in populations, and this information will have broad implications for understanding the evolution of repetitive DNA regions.

174A

The relationship between aneuploidy and chromosome instability in wild yeast

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Duplication of individual chromosomes, or aneuploidy, is generally considered deleterious. The consequences of aneuploidy vary across organisms, but through detailed studies in lab strains aneuploidy has been associated with proliferative defects, proteostasis stress, transcriptional and metabolic signatures and chromosome instability. The effects of aneuploidy in yeast have been well-characterized in laboratory strain W303, however past work from our lab shows that wild yeast strains frequently harbor chromosomal amplifications and have less severe growth challenges or stress responses. Further work from our lab mapped the genetic basis of aneuploidy tolerance in wild yeast to the RNA binding protein Ssd1p, which is defective in W303. In fact, deleting *SSD1* from wild aneuploid strains largely recapitulates the observed growth defects and transcriptional stress response in W303 aneuploid strains. We and others previously noted that cultures of ssd1- aneuploids are rapidly overtaken by euploid revertants. An unresolved question is whether this observation is due to chromosome instability in ssd1- aneuploids or due to stochastic chromosome loss coupled with a dramatically improved growth rate of euploid revertants. To distinguish this, we generated a suite of YPS1009 strains in which each chromosome is duplicated individually. In euploid cells, we integrated a cassette containing a galactose-inducible promoter next to each centromere and induced high levels of transcription, which disrupts kinetochore function and chromosome segregation to produce aneuploid cells. We characterized the fitness costs and dependence on Ssd1p in the suite of YPS1009 aneuploid strains: the fitness cost varies by chromosome and most, but not all, aneuploids require Ssd1p for wild-type growth. To assay chromosome instability in YPS1009 aneuploids, we devised a strategy to determine the rate at which duplicated chromosomes were lost, starting from a single cell progenitor. Our preliminary results suggest that duplicated chromosomes are lost at different rates from one another. For some chromosomes, stability was influenced if the cassette remains next to the centromere. In at least one case, the modified chromosome was lost at a significantly higher rate in ssd1- aneuploids, raising the possibility that Ssd1p has a role in the proper segregation of particular chromosomes.

175A

The INO80 and SWR1 chromatin remodeling complexes function in chromosome segregation and ploidy maintenance

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Faithful chromosome segregation is an essential property of cell division, required for the maintenance of genome integrity. The main goal of this work is to gain an understanding of the role that chromatin and chromatin remodeling complexes have during mitotic chromosome segregation. To identify proteins that are involved in ploidy maintenance, we carried out a screen of the non-essential deletion library for genes that when mutated caused ploidy increase. Among the mutants that increased ploidy, we encountered members of the INO80 and SWR1 complex. Both ATP-dependent chromatin-remodeling complexes participate in a variety of biological processes including transcription, DNA repair and DNA replication. INO80 catalyzes the eviction of the H2A.Z histone variant replacing it with H2A. This complex is comprised of 15 subunits, and their specific contribution to chromosome segregations remains largely unknown. The INO80 complex has been implicated in the maintenance of ploidy through the characterization of mutations of the genes encoding the Ies6 and Ino80 subunits (Chambers et al. doi:10.1101/gad.199976.112), which result in ploidy increase. The SWR1 complex catalyzes the exchange of H2A for H2A.Z. The yeast SWR1 complex is comprised of 14 subunits. The Swr1 subunit creates the scaffold of the complex and is essential for its enzymatic activity. To evaluate the contribution of each subunit to chromosome segregation, we tested deletion mutants of all the non-essential subunits of both complexes and a ts allele of *ARP4* for benomyl sensitivity, ploidy maintenance and chromosome segregation. We also analyzed genetic interactions among subunits of both complexes and characterized *ino80Δ* and *swr1Δ* strains with respect to cell cycle progression and chromosome stability. Our data indicate that both complexes are required for the maintenance of normal ploidy and genomic stability, but only the catalytic subunits and a few other subunits are required for this function, including Arp4. The Ino80 subunit appears to associate with pericentric chromatin even when some subunits that are required for ploidy maintenance are deleted. We are currently assessing whether the presence of Ino80 at pericentromeres correlates with H2A.Z levels and extending these studies to SWR1 complex subunits.

176A

Novel insights into how Eco1p acetylation of Smc3p inhibits Scc2p activation of cohesin

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Sister chromatid cohesion is mediated by an evolutionarily conserved “cohesin complex” composed of 4 subunits, Smc1p, Smc3p, Mcd1p (Scc1p) and Scc3p. Cohesion is established in S phase and maintained through metaphase. Cohesin contains two ATPases formed by heterodimerization of the Smc1p and Smc3p head domains. Both ATPases are required for cohesin to bind DNA. The Scc2p/Scc4p complex is also required for cohesin to bind DNA by further stimulating the intrinsic cohesin ATPase activity. However, this DNA binding is unstable. During S phase, Eco1p (Ctf7p) acetylates Smc3p residue K113 in a subset of cohesin complexes, stabilizing cohesin-DNA binding to enable sister chromatid cohesion. Smc3p acetylation inhibits the ability of Scc2p to stimulate the cohesin ATPase. It is unclear how Smc3p acetylation alters cohesin to make it refractory to Scc2p activation.

Here we conducted a genetic screen to investigate how Smc3p K113 acetylation modulates cohesin complex. We took advantage of the fact the strains bearing the Smc3p acetyl-mimic (Smc3-K113Q) as the sole Smc3p in cells are inviable and are defective in sister chromatid cohesion. We identified spontaneous suppressor mutations that restored viability to *smc3-K113Q* cells. Three suppressors were in Smc1p and three in Smc3p. Three suppressors mapped to Smc head domains. Intriguingly, cryo-EM had identified three places where the cohesin has interfaces with Scc2p, one was near Smc3p-K113. The other two Scc2p interfaces were within either the Smc1p head or Smc3p head domains and our suppressors mapped to each of these interfaces. Our most robust suppressor is in the Smc1p head domain, in close proximity to the Smc3p ATPase, and adjacent to the signature domain known to regulate ATPases. Our data supports a model whereby Scc2p stimulates the Smc3p ATPase to enable cohesin loading onto DNA. Once cohesin is loaded onto DNA, Smc3p acetylation inhibits the ability of Scc2p to activate the Smc3p ATPase and enable cohesion.

177B

Using Mutational Scanning in *Saccharomyces cerevisiae MUS81* to Identify Synthetic Lethal Interactions

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The concept of synthetic lethality (SL) holds great promise for the selective killing of cancer cells. However, after more than 20 years of searching for SL-based anti-cancer therapies, only one example has reached the clinic: PARP inhibitors. Successful PARPi’s “trap” PARP on DNA by disrupting key mechanistic steps required to dissociate from DNA. Trapped PARP-DNA complexes are toxic and require the concerted action of repair factors to facilitate resolution. However, repairing this DNA-protein lesion involves generating double stranded breaks, which, in the absence of key homologous recombination factors, such as in certain cancer cells, compromises genome fidelity leading to trapping-mediated SL. Trapping inhibitors have the potential to be effective even in the presence of uninhibited target, in effect phenocopying a dominant mutation. Conversely, dominant mutations could be used to find new targets that can be trapped and to model the effects of trapping inhibitors.

Other DNA repair proteins have the potential to be trapped by small molecules, which could result in SL and expand the range of cancers that can be targeted by SL. Mus81 is a DNA repair enzyme associated with the increased survival and drug resistance of tumours. As an endonuclease involved primarily in restarting stalled replication forks and resolving late recombination intermediates, transient interactions with DNA are imperative for Mus81 function. Importantly, cells that lack *MUS81* or carry catalytically inactivating mutations often require

other DNA repair enzymes for viability and tolerance to replication stress, highlighting its value as a target for trapping in anti-cancer therapies.

I generated a library of mutant *Saccharomyces cerevisiae MUS81* to screen for mutations that, when expressed in wild-type cells, cause dominant sensitivity to methylmethanesulfonate (MMS), a DNA alkylating agent. Dominant mutations causing hypersensitivity to MMS clustered at the interface of the catalytic domain and show conservation between species. Additionally, expressing these dominant mutants in cells lacking endogenous *MUS81* cause a greater sensitivity to DNA damage than loss of *MUS81*. Expression of dominant *MUS81* mutants in *SGS1*- or *SRS2*-deficient cells causes strong synergistic growth defects and extreme sensitivity to low concentrations of MMS. These observations suggest that increased recombination intermediates, generated by DNA damaging agents or loss of anti-recombinogenic factors such as Sgs1 and Srs2 enhance the toxic effect of the dominant Mus81 mutations.

To determine whether these mutations enhance Mus81 retention on DNA, thereby trapping, biochemical assays will be used to assess DNA-protein stability and catalytic activity. Using this approach, we can map allosteric sites required for Mus81-DNA interactions.

178B

The budding yeast superoxide dismutase Sod2 preserves nuclear genome integrity under oxidative stress

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This study establishes the importance of the mitochondrial superoxide dismutase Sod2 in maintaining nuclear genome integrity under oxidative stress and identifies mechanisms that respond to DNA damage in cells lacking Sod2. While the importance of antioxidant enzymes such as Sod1 and Tsa1 in maintaining genome stability independently and in conjunction with homologous recombination genes is well known, such data for Sod2 is scant. Upon discovering upregulation of Sod2 in cells lacking Sgs1 via our proteomics screen, we followed up with genetic screens like drug sensitivity assays and assays that measure the rate of different types of genome instability to test the influence of Sod2 on nuclear genome integrity and its functional interactions with DNA damage repair/bypass pathways. We show that Sod2 functionally interacts with the helicase Sgs1, strand exchange protein Rad51 and the subunit of DNA polymerase delta Pol32 to suppress hypersensitivity to oxidative stress. Our study reveals a model where in the absence of Sod2, oxidative stress induces gross chromosomal rearrangements that are break-induced-replication and translesion-synthesis-dependent, and mutations at the *CAN1* locus that are exclusively translesion-synthesis-dependent. While Sod1 takes up the mantle of being the primary superoxide scavenger and protector of genome stability in yeast, it is SOD2 (MnSOD) that is exceptionally important in higher eukaryotes as SOD2 disruption is lethal in mice and *Drosophila*, thereby representing the evolutionary selectivity and greater physiological relevance of MnSOD. Our findings in yeast bear profound implications for Sod2 in preventing genome instability, which is a hallmark of most cancers in higher eukaryotes, thereby elucidating the ambiguous role of MnSOD in tumor suppression.

179B

Mechanisms of Rad5-mediated mutagenic repair of DNA gaps

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Upon replication stress, cells utilize the post-replication repair pathway to repair single-stranded DNA (ssDNA) and maintain genome integrity. This process is divided into two branches: error-prone translesion synthesis (TLS), signaled by PCNA mono-ubiquitination (PCNA-Ub), and error-free template switching, signaled by PCNA poly-ubiquitination. In *S. cerevisiae*, Rad5 is involved in both branches of repair. When the PCNA poly-ubiquitination functionality of Rad5 is disrupted, Rad5 recruits the TLS polymerase Rev1 to stalled replication forks, resulting in a mutagenic repair of ssDNA in the absence of DNA damage. Details of the how this mutagenic repair is carried out, as well as the relationship between Rad5-mediated TLS and the canonical, PCNA-Ub-mediated TLS, remain to be understood. We found that Rad5-mediated TLS requires the TLS polymerase ζ and does not require Rad30 or the catalytic activity of Rev1. Furthermore, we showed that Rad5-mediated TLS is independent of the physical interaction between Rev1 and PCNA-Ub, and is separable from PCNA-Ub-mediated TLS. In the absence of error-free template switching, both modes of TLS contribute additively to mutagenic ssDNA repair and replication stress response. Collectively, our results highlight the importance of Rad5 in regulating spontaneous mutagenesis in *S. cerevisiae* through different modes of post-replication repair.

180B

Using single-cell tracking to define DNA damage response heterogeneity

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Phenotypic heterogeneity is the existence of non-uniform phenotypes within an isogenic population. In several contexts, phenotypic heterogeneity is an accepted mechanism for the creation of alternate phenotypes, without genetic modification, allowing for increased population fitness to environmental change. While many aspects of DNA damage responses are well characterized, there is a lack of insight into the extent, the origin, and the effect on cell survival, of DNA damage response heterogeneity. Phenotypic heterogeneity, in previous literature, is primarily explored and associated with gene expression variations, while protein localization has largely remained untested. Protein relocalization in response to treatment with genotoxic stress is a hallmark of the DNA damage response. Protein location is an important element of function; therefore, it serves as a good test case to assess the functional impact of response heterogeneity. Previously catalogued proteins, which demonstrate relocalization in response to genotoxic stress, were assembled and interrogated for heterogeneity in localization response to genotoxic treatment with the DNA alkylating agent MMS. Protein relocalization was analyzed by imaging cells expressing these GFP-tagged proteins on microfluidic chips at the resolution of single-cell trajectories. Protein localization changes were

quantified in individual cells over time by measuring changes in corresponding fluorescence intensity distributions. The methods developed allow for protein localization dynamics to be measured for each cell, and for phenotypic penetrance to be calculated along the time course. We determined that proteins involved in the DNA damage response exhibit varying levels of heterogeneity. In some cases, the protein-specific responses can be associated with theoretical schemas such as “bet-hedging.” This work, for the first time, characterized protein relocalization heterogeneity in the eukaryotic DNA damage response context, in individual single cells followed over time. The results also emphasize the importance of single-cell-tracking for determining DNA damage response variation and gaining a more robust measurement of penetrance. The complete picture of DNA damage response heterogeneity that emerges will be an important foundation for associating heterogeneity with functional outcomes.

181B

Engineering yeast to combat cosmic radiation: a new trajectory to study DNA damage and genome integrity Hamid Gaikani, Guri Giaever, Corey Nislow Faculty of Pharmaceutical Sciences, University of British Columbia

How cells respond to DNA damage and maintain the stability of their genome have been long-standing questions since the discovery of DNA, and for much of this research, yeast has been a pathfinder model organism. Another fruitful line of inquiry to understand these mechanisms is to identify and study organisms that can thrive in conditions that would otherwise destroy DNA, so-called extremophiles. Tardigrades are one of the most resilient eukaryotic organisms known—showing an astonishing tolerance against environmental stresses, including DNA damaging agents. One of the key players of such extreme tolerance is a tardigrade-specific protein known as **D**amage **su**ppressor **p**rotein (Dsup) for which no homologs exist outside of tardigrades. Human cells in culture, modified to express Dsup protein, had a significant increase in survival to X-ray damage versus unmodified controls. Nevertheless, little is known regarding the Dsup’s mechanism of action in the cell nor its potential interactions with the host genome. Hence, we sought to leverage “the awesome power of yeast genetics” to further characterize its behavior in cells when exposed to either radiation- or chemically-induced DNA damage. Specifically, we have engineered Saccharomyces cerevisiae to express Dsup and will combine two of these techniques —Synthetic Genetic Array (SGA) and Synthetic Dosage lethality (SDL)—with pooled screening (aka chemogenomics) to systematically decipher genes and pathways that might interact with Dsup. Since little is known regarding the potential enzymatic activity of Dsup, SDL, a technique well-suited to identify enzyme substrates, may be particularly informative. Moreover, we are phenotyping Dsup-expressing cells in diverse environmental stresses (such as drugs, desiccation, high osmolarity, etc.) to determine if exogenously expressed Dsup can ameliorate the effect of these perturbations. The results of this study can provide insights into genome integrity and cancer radiotherapy, and furthermore, the lessons learned in yeast can apply to genome engineering of organisms for long-term missions beyond lower earth orbit.

182B

***pol30* mutants through two pathways to dissociating Srs2 to rescue DDT defects** Li Fan Biochemistry, microbiology and immunology, University of Saskatchewan

In *Saccharomyces cerevisiae*, DNA-damage tolerance (DDT) pathway is specifically to bypass replication-blocking lesions to guarantee genome integrity. DDT is mediated by sequential PCNA (encoded by *POL30*) ubiquitination and sumoylation at K164. Monoubiquitinated PCNA by Rad6-Rad18 facilitates translesion DNA synthesis. While PCNA can be further polyubiquitinated by Mms2-Ubc13-Rad5, which promotes error-free DDT. Furthermore, Pol30-K164 can be sumoylated which helps to recruit Srs2 to prevent salvage homologous recombination (HR). Srs2 through its atypical PCNA interaction protein (PIP) box interacts with PCNA and symoylation interaction motif (SIM) interacts with SUMO, therefore recruited by SUMO-PCNA. Deletion of *RAD6*, *RAD5* or *RAD18* results in severe DNA-damage sensitivity, which can be rescued by deletion of *SRS2* most probably through activating salvage HR. But the mechanism was not identified. In my study, it was found that one of the DNA-damage resistant mutants from *rad5*Δ cells include a *pol30-A171D* mutation, which could rescue both *rad5*Δ and *rad18*Δ DNA-damage sensitivity in a *srs*2-dependent and PCNA sumoylation-independent manner. Pol30-A171D abolishes physical interaction with Srs2 but not another PCNA-interacting protein Rad30; however, Pol30-A171 is not located in the PCNA-Srs2 interface. The SUMO-PCNA-Srs2 structure was analyzed to design and create mutations in the complex interface, one of which, *pol30-I128A*, results in phenotypes reminiscent of *pol30-A171D*. In addition, *pol30-A171D* is synergistic with *pol30-K164R* in the rescuing and the *srs2* mutation is epistatic to both *pol30* mutations. Hence, yeast cells dissociate Srs2 from PCNA to rescue DDT defects probably through two pathways one is through reducing physical interaction with Srs2, like *pol30-A171D* or *pol30-I128A*; the other is through affecting PCNA sumoylation, like *pol30-K164R*.

183B

Fork reversal activity of Rad5 and the helicase activity of Rrm3 interact in the prevention of DNA double-strand breaks in yeast Julius C Muellner, Kristina Schmidt Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida

The genome must be monitored constantly, especially during DNA replication, to ensure its duplication is accurately completed and in a timely manner to prevent genome instability. In *Saccharomyces cerevisiae* a member of the PIF1 family, the 5’ to 3’ DNA helicase Rrm3, facilitates replication fork progression. The deletion of Rrm3 or the disruption of its helicase activity leads to an increase in paused replication forks at more than 1,000 sites in the genome, including ribosomal DNA repeats, tRNA genes, centromeres, telomeres, and the silent mating-type loci. We are using *Saccharomyces cerevisiae* deficient of Rrm3 as a model organism to identify how cells deal with increased stalled replication forks. In response to increased stalled replication forks we observe an enrichment of Rad5, a member of the SWI/SNF family of ATPases, at the chromatin. Here we are further characterizing the negative genetic interaction between *RRM3* and *RAD5*. We determine that Rad5 activities involved in replication fork reversal, which are conferred by its DNA helicase and HIRAN DNA-binding domains, are needed to tolerate DNA damage and replication stress in the absence of Rrm3. In contrast, the

ubiquitin-ligase activity of Rad5, which is conferred by its RING domain and involved in DNA-damage-induced poly-ubiquitination of PCNA and DNA lesion bypass by homology-mediated template switching, is not required. Furthermore, by using fluorescence microscopy we observed increased Rad52 foci, indicating increased double-strand breaks, in *rrm3*Δ*rad5*Δ *cells* under DNA replication stress. We determine that Rrm3's ATPase/helicase activity, but not its N-terminus are required to suppress double-strand break formation in the absence of Rad5, whereas Rad5's fork reversal activities are needed in cells deficient of Rrm3. Besides Rad5-mediated fork reversal, Rrm3-dependent stalled replication forks also provide a substrate for the endonuclease Mus81, as in its absence we observe synergistic increase in double-strand break formation and genome rearrangement. Thus, if blocks to replisome progression cannot be overcome due to the absence of Rrm3, our findings indicate two pathways, Rad5-mediated fork reversal and Mus81-mediated fork cleavage for fork rescue.

184B

Genetically engineering a strain of *S. cerevisiae* with a single circular chromosome

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While prokaryotes and archaea generally contain a single circular chromosome, eukaryotes like yeast contain multiple linear chromosomes. Linear chromosomes require telomeres at the ends to protect the chromosome from degradation and spurious recombination. Additionally, linear chromosomes can't be fully copied during DNA replication. To get around this end-replication problem, most eukaryotes require the ribonucleoprotein enzyme complex telomerase to maintain telomere length. Even with telomerase, telomeres shorten with organismal aging and telomerase expression is upregulated in >85% of human cancers. Since telomeres create such issues for cells, our lab is interested in why linear chromosomes evolved in the first place. To experimentally address this question, our lab has developed a genetic engineering approach to create circularized versions of each of the 16 *Saccharomyces cerevisiae* chromosomes. Briefly, we use PCR to create DNA cassettes to insert into the right and left arms of a chromosome. Each cassette contains: 1) a unique selectable marker (*HIS3* or *LEU2*), 2) one half of the *URA3* gene with a region of overlapping homology, and 3) homology to the telomeric region of the target chromosome arm. The cassette DNA is transformed into haploid yeast cells, and integration is selected for by growth on media lacking histidine and leucine. To verify the cassettes integrated into the expected location, we perform PCR across the integration junction. Once double integrants are confirmed, DNA recombination between the two *URA3* halves will result in circularization of the chromosome and allow growth on media lacking uracil. To date, our lab has successfully obtained two circular chromosomes (Ch. IV and VIII), providing proof-of-principle that our approach can work. I have chosen a different chromosome, and am currently building the cassettes to circularize a new chromosome. Successful completion of circularization will allow us to characterize these strains relative to wild-type to better understand how circular versus linear chromosome architecture is tolerated in a eukaryotic species.

185B

Investigating the role of post-replication repair in the resolution of transcription-associated replication stress.

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Precise DNA replication is crucial for genome integrity. This process is constantly challenged by endogenous and exogenous stressors, which in turn hinder the synthesis of daughter strands and stall replication fork progression. The post-replication repair (PRR) is a DNA damage tolerance mechanism that acts at stalled forks to ensure replication completion. The PRR pathway can be further broken down into two branches – Translesion synthesis (TLS) and Template switching (TS) – that are activated by mono- or poly-ubiquitination of the processivity factor PCNA in a Rad18-dependent manner. However, the regulation of the two branches is not fully understood. Here, we use *Saccharomyces cerevisiae* as a model to investigate the role of PRR in the resolution of replication stress driven by transcription-replication conflicts and R-loops, hybrids of DNA and RNA which have been shown to accumulate in PRR mutants in yeast and human cells. We observed that disruption of both PRR pathways in a Rad18 deletion background leads to the accumulation of exposed ssDNA. Using a plasmid system that induces transcription-replication conflicts we also saw an increased frequency in recombination that is reduced with overexpression of the R-loop processing protein RNase H1. We further explore this relationship by generating a series of PRR mutants combined with either RNase H1, RNase H2 or both, and also use a non-ubiquitylatable mutant PCNA strain to verify ubiquitination dependency. By unraveling interactions of the genes within PRR and their potential roles in reducing transcription-replication conflict and R-loop associated DNA damage, we aim to reveal new ways that mutagenesis occurs during cancer formation.

186B

Contributions of Fumarase and the Histone Variant H2A.Z during Responses to DNA Damage and DNA Replication Stress

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Fumarase is a well-characterized TCA cycle enzyme that catalyzes the reversible conversion of fumarate to malate. In mammals, fumarase acts as a tumor suppressor, and loss-of-function mutations in the FH gene in hereditary leiomyomatosis and renal cell cancer result in the accumulation of intracellular fumarate—a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Here, we report that *Saccharomyces cerevisiae* fumarase acts as a response factor for DNA damage and DNA replication stress, and fumarate enhances survival of yeast lacking Htz1p. Our findings indicate in response to DNA damage and DNA replication stress, fumarase becomes upregulated and enriched in the nucleus. Fumarase's metabolic product fumarate suppresses the sensitivity to DNA replication stress of *htz1* mutants by inhibiting the H3 K4-specific histone demethylase Jhd2p, thereby increasing H3 K4 methylation. While the timing of intra-S phase checkpoint activation and deactivation are largely unaffected by fumarate, sensors and mediators of the DNA Replication Checkpoint, which senses intact stalled replication forks, are required for fumarate-dependent resistance to replication stress in *htz1* mutants. We will present impacts of fumarase and Htz1p on replicative intermediates as well as on alternate pathways for double

stranded DNA break repair, including different sub-pathways of non-homologous end joining. Together, our findings imply metabolic enzymes and metabolites aid in processing sites of DNA damage by affecting chromatin, thereby promoting genome integrity.

187V

G1-Cyclin2 (*CLN2*) promotes chromosome hyper-condensation in *eco1/ctf7 rad61* null cells during hyper-thermic stress in *Saccharomyces cerevisiae*

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Eco1/Ctf7 is a highly conserved acetyltransferase that activates cohesin complexes and is critical for sister chromatid cohesion, chromosome condensation, DNA damage repair, and gene transcription. Mutations in the human homolog of *ECO1* (*ESCO2/EFO2*), or in genes that encode cohesin subunits, result in severe developmental abnormalities and intellectual disabilities referred to as Roberts Syndrome (RBS) and Cornelia de Lange Syndrome (CdLS), respectively. In yeast, deletion of *ECO1* results in cell inviability. Co-deletion of *RAD61 (WAPL* in humans), however, produces viable yeast cells - but only within a narrow temperature range. Thus, Eco1 responds to hyper-thermic stress through a mechanism that appears regulated independent of Rad61. Here, we report that the G1 cyclin, Cln2, antagonizes Eco1 function such that *eco1 rad61 cln2* triple mutant cells exhibit robust growth over a broad range of temperatures, similar to wildtype cells. While Cln1, Cln2 and Cln3 are functionally redundant G1 cyclins, neither *CLN1* nor *CLN3* deletions rescue the temperature-sensitive growth defects of *eco1* rad*61* double mutant. We further provide evidence that the suppression provided by *CLN2* deletion occurs independent of START but instead may act through alterations in chromosome condensation. These findings reveal that Cln2 promotes chromatin hyper-condensation, an activity that is unique among the G1 cyclin family.

188V

Ortholog replacement reveals a novel function of the transcription factor TFIIIC complex in mitotic chromosome segregation

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Recent studies have shown that altered transcriptional regulation plays a crucial role in many adaptive evolution events. However, the extent of these changes in transcription factors that derive evolution remains understudied. We investigated whether essential transcription factors (eTFs) can also be changed over short evolutionary timescales and how they changed. We observed several orthologous eTFs from other yeast species that could not fully complement the mutants in *Saccharomyces cerevisiae*. Our results suggest that eTFs such as Tfc7 have changed their functions or interactions to a certain level to become partially incompatible between species. Tfc7 is a subunit of the TFIIIC complex which transcribes tRNA genes with the help of RNA polymerase III. In the orthologous Tfc7-replacement (*Kl-TFC7*) line, we observed that the general expression of tRNAs was not affected in the population, even though, the TFIIIC complex shows lower enrichment at tRNA genes. In addition, Tfc1 a direct interactor of Tfc7 in the TFIIIC complex shows no interaction with KlTfc7 in the *Kl-TFC7* line. Furthermore, deletion of the spindle checkpoint gene (Mad2) increased the incompatibility, suggesting that chromosome-spindle attachments might be compromised in the *Kl-TFC7* line. Upon further investigation, a significant chromosome segregation defect was observed in the Kl-TFC7 population, along with a significantly elongated cell cycle. Coincidentally, TFIIIC has been shown to recruit and stabilize cohesin and condensin on chromosomes at tRNA genes during interface and mitosis. We propose a model to explain how Tfc7 has changed between two yeast species. We speculate that chromosome missegregation is caused by abnormal cohesion-condensin loading in the replacement lines, because of unstable TFIIIC complex formation.
Keywords: essential transcription factor, evolution, Tfc7, TFIIIC, cohesion, condensin, tRNA genes

189V

A non-canonical Dun1 FHA domain interaction surface contributes to ligand specificity and resistance to genotoxic stress

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DNA damage Uninducible (Dun1) is a cell cycle checkpoint kinase involved in regulating dNTP synthesis. In the budding yeast *Saccharomyces cerevisiae*, Rad53-dependent phosphorylation of Dun1 leads to the phosphorylation of downstream factors involved in the regulation of dNTP levels. Dun1-dependent phosphorylation of Dif1 and Sml1 targets them for degradation, thereby removing their inhibitory controls on the assembly and function of the ribonucleotide reductase (RNR) holoenzyme. We examined the involvement of a non-canonical lateral binding surface on the Dun1 FHA domain, distinct from its recognition region for phosphothreonine (pThr)-containing epitopes, in mediating Dif1 and Sml1 interactions as well as the consequent contribution to cell growth and resistance to genotoxic stress. Yeast two-hybrid assays revealed that the Dun1 FHA domain is necessary for both interactions with Dif1 and Sml1. However, the Dun1 FHA domain is not sufficient for the full-strength interaction with Dif1 or the interaction with Sml1. Bioinformatics analysis was used to identify candidate interaction residues on the Dun1 FHA domain lateral surface, and corresponding site-directed mutagenesis was used to alter these residues. Candidate residues were selected based on sequence conservation, as well as the position, size, and charge of the side chains. Yeast two-hybrid assays revealed that Dun1 interacts with Dif1 primarily through the Dun1 FHA pThr-epitope binding site whereas the Dun1-Sml1 interaction requires both the Dun1 FHA pThr-epitope binding site and the non-canonical lateral binding surface. Spot plate assays revealed that disruption of the Dun1 lateral interaction surface and pThr-epitope binding site resulted in sensitivity to hydroxyurea, bleomycin and phleomycin, indicating that Dun1 checkpoint function, including dNTP level regulation, relies on the protein-protein interactions mediated by both regions of the Dun1 FHA domain.

190V

The absence of *SAF1* and *CTF8* together contributes to MMS Resistance and HU Sensitivity in S*. cerevisiae* Narendra K Bairwa, Meenu Sharma, V Verma Shri Mata Vaishno Devi University

The alternative replication factor C complex, non-essential for cell viability, consisting of three subunits: Ctf18, Ctf8, and Dcc1 is involved in sister chromatid cohesion and assists the loading of PCNA onto the chromosome. *CTF8* null mutants in *S.cerevisiae* show chromosome instability and a high frequency of chromosome loss. Saf1, an F-Box protein, is involved in proteasome-dependent degradation of Aah1p, the Adenine deaminase. In this study, we have investigated the binary genetic interaction between the *SAF1* and *CTF8* genes. Yeast strains containing single- and double-deletions of the two genes were evaluated for growth fitness, genome stability, and response to genotoxins, hydroxyurea (HU), and methyl methanesulfonate (MMS). The absence of *SAF1* and *CTF8* together shows an increased growth rate in comparison to single mutants of both gene and parental strain on the YPD medium. However, the ***saf1Δctf8Δ*** strain showed resistance to MMS, sensitivity to HU, elevated rate of Ty1 retro-transposition, and altered nuclear status. Based on the observation we suggest that the mechanism of differential growth phenotype due to loss of *SAF1* and *CTF8* together in the presence of genotoxic stress indicates gene-gene interaction in the regulation of growth and cellular response to stress agents.

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191V

The absence of F-box motif Encoding Gene *SAF1* and Chromatin Associated factor *CTF8* together contributes to MMS Resistance and HU Sensitive phenotype in *S. cerevisiae* Meenu Sharma School of Biotechnology, Shri Mata Vaishno Devi University

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Cohesion related replication factor-C complex constitutes, three subunits called Ctf18, Ctf8 and Dcc1. These three subunit complex assist the loading of PCNA onto the chromosome. None of the replication factor C components are essential for cell viability. The null mutant of *CTF8* in *S.cerevisiae* shows high frequency of chromosome loss. The *SAF1* gene product of *S. cerevisiae*involves in recruitment and degradation of adenine deaminase factor Aah1p through SCF-E3 ligase mediated ubiquitination. Here we have investigated the genetic interaction between *SAF1*and *CTF8* genes. The single and double gene deletions of *SAF1* and *CTF8* were constructed in BY4741 genetic background and evaluated for growth fitness, genome stability,and cellular growth response to genotoxic stress caused by hydroxyurea (HU) and methyl methane sulfonate (MMS). The ***saf1Δctf8Δ*** strain showed the increased growth phenotype in comparison to WT, *saf1Δ*and *ctf8Δ* strains on YPD medium. However ***saf1Δctf8Δ*** strain when grown in the presence MMS showed resistance and HU sensitive phenotype when compared with *saf1Δ, ctf8Δ.* The frequency of Ty1 retro-transposition was also elevated in ***saf1Δctf8Δ*** in comparison to either *saf1Δ* or *ctf8Δ.* The number of cells showing the two or multi-nuclei phenotype was also increased in ***saf1Δctf8Δ*** cells when compared with the either *saf1Δ* or *ctf8Δ.* Based on these observations, we report that the absence of both *SAF1* and *CTF8* genes together contributes to MMS resistance, HU sensitivity, and genome instability phenotype. This report warrants the further investigation into the mechanisms of differential growth phenotype due to loss of *SAF1* and *CTF8* together in presence of genotoxic stress.

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192V

Enhanced mutagenicity during meiosis: the involvement of DNA repair and recombination genes Ayelet Arbel-Eden[1,21] Medical Laboratory Science, Hadassah Academic College, [2]Genetics, Alexander Silberman Institute of Life Sciences The Hebrew University

Meiosis is a unique cell division, by which diploid cells produce haploid gametes. A major feature of meiosis is the formation of new combinations of genetic material by recombination and independent assortment of chromosomes. Mutations that occur during meiosis further increase variation. Mutation rates in yeast meiosis are at least 7-fold higher than in mitotic cell divisions, as determined by the *CAN1* reporter gene. Meiotic mutations appear at the same time and by the same kinetics as recombination events, and firmly depend on Spo11-induced DNA double-strand breaks (DSBs). We examined mutations occurrence in the context of homologous recombination, by monitoring mutation rates in strains singly deleted for *mre11*, *dmc1*, *rad54*, *tid1* and other genes, which affect various stages along the meiotic recombination process.

We find that 43% of meiotic mutations are associated with crossing over events nearby, and suggest that the remaining mutations may result from sister-chromatid repair of meiotic DSBs. By examining meiotic mutagenicity in haploid strains (where repair of meiotic DSBs must occur through interaction with the sister chromatid) we show that the occurrence of meiotic mutations depend on DSBs, does not require the presence of non-sister chromatids, and the spectra of meiotic mutations in haploids and in diploids are similar.

In search for the molecular basis for the elevated mutagenicity in meiosis, we find that Trans-Lesion DNA polymerases are not the source of the enhanced mutagenicity in meiosis. Currently, we are examining replicative polymerases, such as Pol-delta and Pol-epsilon, to inquire whether mutagenicity may arise from their involvement in DSB repair during meiosis.

Mismatch correction play an important role in maintaining normal genome mutation rates. We find unique and surprising contribution of mismatch repair proteins to meiotic mutagenicity.

As to the evolutionary impact of meiotic mutagenicity, it should be emphasized that the new, rare meiotic mutations affect the germline, transmitted to the next generation and may contribute to long-range evolutionary processes and enhance adaptation to challenging environments.

193A

A noncanonical GTPase signaling mechanism controls exit from mitosis in budding yeast

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In budding yeast, exit from mitosis is coupled to nuclear/spindle position to ensure successful genome partitioning between mother and daughter cell. This coupling occurs through a GTPase signaling cascade known as the mitotic exit network (MEN). Only when the anaphase spindle is positioned correctly along the mother-bud axis is the MEN activated to promote exit from mitosis. The MEN senses spindle position via a Ras-like GTPase Tem1. How the GTP/GDP cycle of Tem1 translates the status of spindle position to the activation of its effector protein Cdc15 is not fully understood. Tem1 primarily localizes to the spindle pole body (SPB) that migrates into the bud during anaphase. Here, we show that the nucleotide state of Tem1 dictates its SPB localization. More importantly, by artificially tethering Tem1 to the SPB, we demonstrate that the essential function of Tem1-GTP is to localize Tem1 to the SPB. Localization to the SPB mainly functions to concentrate Tem1, as we could bypass this essential localization by concentrating Tem1 in the cytoplasm with genetically encoded multimeric nanoparticles (GEMs). With additional data on the GTPase cycle and localization dynamics of Tem1, we propose a new model for spindle position sensing by the MEN. Overall, our study reveals a distinct localization/concentration-based GTPase signaling mechanism for Tem1 that differs considerably from the canonical Ras-like GTPase signaling paradigm, in which the nucleotide state of the GTPase regulates effector binding and activation.

194A

Why is the yeast lag phase so long?

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Microorganisms commonly face environments with dynamic nutrient availability. This makes it crucial for an organism's fitness to appropriately respond to the environmental change, since each metabolic response requires an investment of energy, time and precious building blocks. In many cases, such metabolic rewiring is accompanied by a period of delayed growth, often referred to as a lag phase. The duration of this lag phase can vary significantly between organisms, environments and phenotypes. Especially between members of the same species, and more strikingly even within isogenic populations, large natural variation in lag duration exists. This raises a number of questions: Why would an organism have a long lag phase? Are there any benefits to having a longer lag phase? Which factors determine lag phase length? How is the lag phase tuned, and which genetic factors underlie its natural variation?

In this project, we use the yeast *Saccharomyces cerevisiae* as a model system, specifically focusing on its response to an environmental switch from glucose to a secondary carbon source. Genome-wide screens suggest that inducing carbon source-specific genes such as transporters and hydrolases, though necessary, is not the rate-limiting process determining lag phase duration upon a carbon source shift. Rather, re-routing the carbon flux from fermentation to respiration seems to be the major bottleneck for how fast cells adapt to a nutritional change. QTL analysis suggests that natural variation in the uncharacterized gene *YLR108C* is at the heart of strain-specific lag duration differences. Moreover, this gene seems to encode a key fine-tuner of the lag phase, most likely through regulation of respiration. Furthermore, swapping *YLR108C* alleles shows that a short lag phase goes hand-in-hand with slower growth in stable environments.

Together, our results show that the lag phase is a complex phenomenon influenced by both genetic and epigenetic mechanisms coordinating the respiration-fermentation balance to obtain optimal fitness in either stable or fluctuating environments.

195A

K29-linked unanchored polyubiquitin chains at crossroads with the functional wiring between ribosome biogenesis and cell division

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Ubiquitination regulates key aspects of cellular physiology by regulating protein degradation and activity status. Deubiquitylases (DUBs) modulate ubiquitin signaling by editing or en bloc removal of polyubiquitin chains on substrates attached by Ubiquitin (Ub) E3 ligases releasing unanchored polyubiquitin chains. In this study, we uncover a complex functional interplay between E3 Ub ligases Ufd4 and Hul5, and DUBs Ubp2 and Ubp14, in synthesis and recycling respectively, of unconventional K29-linked unanchored polyubiquitin chains contributing to our larger fundamental understanding of Ub homeostasis. We report accumulation of K29-linked unanchored polyubiquitin chains into inclusions and their resulting gain-of-function toxicity to be manifested in part through their association with maturing ribosomes to adversely affect their assembly activating the Ribosome assembly stress response (RASTR) which forms the underlying trigger for sequestration of cellular factors including ribosomal proteins and Anaphase promoting complex (APC) at Intranuclear Quality control compartment (INQ).We further find that APC's sequestration at INQ is consistent with its involvement in ribosome biogenesis and coincides with defects in mitosis, thereby providing a potential cell cycle checkpoint like mechanism for slowing mitosis during defective ribosome assembly by sequestering APC at INQ. The finding thus highlights an intricate functional wiring between Ub homeostasis, ribosome assembly and cell cycle. Importantly, APC's sequestration seems to be conserved from yeast to humans and provides an exciting perspective for therapeutic interventions into Ribosomopathies.

196A

Regulation of Cdc42 Protein Levels Impacts a Cell Differentiation Program in Yeast

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Rho GTPases are central regulators of cell polarity and signaling. Although many aspects of Rho GTPase regulation are understood, how these proteins are directed to function in certain settings remains unclear. We show here that the protein levels of the conserved yeast Rho GTPase Cdc42p are regulated, which impacts a subset of its biological functions. We discovered that Cdc42p is ubiquitinated, and the active (GTP-bound) conformation of the protein is turned over by the NEDD4 E3 ubiquitin ligase Rsp5p, the DnaJ/HSP40 chaperone, Ydj1p, and the HSP70 chaperone Ssa1p in the 26S proteosome. A GTP-locked (Q61L) and turnover-defective (TD) version of Cdc42p, Cdc42p$^{Q61L+TD}$, hyperactivated the MAPK pathway that regulates filamentous growth (fMAPK). Unexpectedly, Cdc42p$^{Q61L+TD}$ did not influence the activity of another Cdc42p-dependent MAPK pathway (Mating) that shares components with the fMAPK pathway. The fMAPK pathway adaptor, Bem4p, stabilized Cdc42p protein levels, which resulted in sustained fMAPK pathway signaling. Our results identify Cdc42p turnover regulation as being critical for the regulation of a MAPK pathway. The control of Rho GTPase levels by stabilization and turnover may be a general feature of signaling pathway regulation, which can result in the execution of specific developmental programs.

197A

PAS kinase controls cellular NADP(H) levels through the phosphorylation and regulation of NAD kinase

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Protein kinases are involved in controlling many metabolic pathways by phosphorylating and regulating the protein substrates in the cells, upregulating and downregulating disparate pathways in concert to facilitate adaptation to environmental conditions. Dysregulation of these metabolic pathways may lead to the disruption of critical cellular processes such as growth, respiration, and lipid biogenesis, thereby contributing to the development of diseases such as cancer. PAS kinase, conserved in yeast and mammals, senses cellular glucose levels and regulates central metabolism accordingly. Our lab has identified five PAS kinase substrates, Cbf1, Mot3, Pbp1, Zds1 and Utr1 (the human homologue is NAD kinase), which play an important role in maintaining cellular respiration, lipid biogenesis, glucose homeostasis and cellular energy metabolism in cells. The focus of this study is to better characterize the effects of PAS kinase-dependent phosphorylation of NAD kinase, the sole cellular source of NADP(H). NADP(H) is required for over 300 reactions in the cell including macromolecular biosynthesis reactions (nucleotide, proteins and fatty acids) as well as reactions that neutralize reactive oxygen species (ROS) produced as a result of high metabolic activity, such as in highly proliferating cancerous cells. Herein, I present the phosphorylation and regulation of NAD kinase by PAS kinase in yeast cells, providing evidence for its phosphorylation in vitro as well as the regulation of its activity and NAD(P)(H) levels in vivo. In addition, the phosphorylation of NAD kinase is shown to affect downstream processes such as cellular growth rates and ROS levels. The control of NAD(P)(H) levels by PAS kinase is another pathway by which PAS kinase controls central cellular metabolism, the dysregulation of which lies at the heart of several diseases including cancer.

198A

Identifying the features of highly diverged Wtf proteins to elucidate the mechanism of action

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Meiotic drivers are selfish genes that unfairly influence gametogenesis to increase their transmission into the offspring. The *wtf* meiotic driver was first discovered in the recently diverged fission yeast species *Schizosaccharomyces pombe*. These gamete-killing *wtf* genes drive by encoding both a poison protein (Wtf^{poison}) and an antidote (Wtf^{antidote}) protein. In heterozygous diploids, the Wtf^{poison} targets all spores (yeast gametes) during gametogenesis, while only the spores carrying the *wtf*+ allele are rescued by the Wtf^{antidote}. Recent work has identified homologous *wtf* drivers across diverse fission yeast species *S. octosporus, S. osmophilus,* and *S. cryophilus*. These diverse *wtf* genes are estimated to have diverged over 100 million years ago from *S. pombe* . In this work, we aim to identify the key features of other highly diverged Wtf proteins that are relevant to their function. The results of this work will elucidate if the highly diverged *wtf* genes are functionally similar, and further the understanding of the mechanism of *wtf* drive in fission yeast.

199A

Exploring alternate start sites for a gene of unknown function in the yeast *Saccharomyces cerevisiae*

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The *Saccharomyces cerevisiae* ORF *YER066W* (*RRT13*) is a WD40 repeat containing gene of unknown function. Previous work has suggested that it may be somehow involved in the process of transposition, possibly by assisting in Gag transport. A significantly higher prevalence of Gag foci has been observed in a *YER066W* deletion strain compared to the wildtype. *YER066W* also has a high degree of homology with *CDC4*, a cell division cycle regulator. Based on comparisons with other yeast species, it is possible that the annotation for *YER066W* should be extended in the 5' direction in the strain S288C. BLAST revealed multiple *YER066W* variants across *S. cerevisiae* strains. Further bioinformatic analysis revealed potential alternative, upstream start sites for the gene, as well as a premature stop upstream of the annotated start. *YER066W* was deleted at the alternate start sites, and growth of wildtype, annotated deletion, and alternative deletions was assayed on YPD. Deletion of *YER066W* at the upstream ATG resulted in significantly increased growth, suggesting that the upstream sequence may have a functional role, possibly in an inhibitory capacity. Gene sequencing showed that the premature stop codon is, in fact, present and not a previous sequencing error. Endogenous transposition assays on wild type and deletion strains showed no significant differences, indicating that *YER066W* is not involved in transposition. *YER066W* involvement in transport was investigated by growing *YER066WΔ* strains carrying plasmids bearing Gag:*URA3*, *CPY1*:*URA3*, or *PHO8*:*URA3* fusions on 5-FOA. *YER066WΔ* strains with Gag:Ura3p grew better than wild type on 5-FOA, suggesting that deleting the ORF causes Gag to be sequestered, which could indicate a role in transport. *YER066WΔ* strains harboring *CPY1*:*URA3* were also able to grow slightly on media lacking uracil and showed slightly reduced growth on 5-FOA, implicating the gene as possibly having a role in the posttranslational ER translocation pathway.

200A

Investigating Kin4-independent functions of Lte1, a Mitotic Exit Network Activator

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Errors in mitotic cell division can result in the development of aneuploid cells that contain an improper amount of genetic material. Although aneuploidy may promote the development of cancer, organisms have developed mechanisms to prevent this outcome. One such mechanism is the Mitotic Exit Network (MEN) found in *S. cerevisiae*, which is a Ras-like signal transduction pathway. Upstream regulation of the MEN begins with the small GTPase Tem1, which functions to activate downstream MEN kinases upon activation. Although the role of the Bub2-Bfa1 complex as a GTP activating protein (GAP) for Tem1 has been well established, the presence of a guanine nucleotide exchange factor (GEF) has yet to be established. The MEN activator Lte1 is known to activate Tem1 indirectly by inhibiting Kin4, an activator of Bfa1. However, recent studies using the *lte1ΔEcoRI* allele of Lte1 have uncovered Kin4-independent Lte1 activities. Due to its sequence homology with other known GEFs, we hypothesize that Lte1 additionally acts as a GEF for Tem1, and that residues within predicted helix B of the Lte1 C-terminus are important for their interaction. In order to assess this hypothesis, mutations were made via site-directed PCR mutagenesis in regions of both *TEM1* and *lte1ΔEcoRI* that are predicted to be important for GEF-GTPase interactions. In Tem1, residues K59 and F82, predicted to prevent GEF interaction based on Ras-family homology, were targeted. In Lte1ΔEcoRI, alpha-helix B, a region shown to be important for Sos and RasGRF GEF activity, was mutated. The activities and proper cellular localization patterns of the resulting mutant proteins were then analyzed. We found that mutations in residues K59 and F82 within Tem1 did not compromise the function or normal localization of the protein to spindle pole bodies (SPBs) in anaphase. In contrast, mutations across the *lte1ΔEcoRI* helix B impacted the protein's structure, function, and localization to the bud cortex. In particular, N687T resulted in a stable protein that could localize correctly, but only exhibited partial mitotic exit function in multiple assays. Our data demonstrate that if Tem1 interacts with a GEF, it likely does so distinctly from Ras. Importantly, we identified Lte1ΔEcoRI-N687T, the first known mutant allele of Lte1 that does not inhibit Kin4, localizes to the bud correctly, but exhibits an impaired ability to activate mitotic exit. This residue may reveal an as yet uncharacterized GEF function for Lte1.

201A

Characterizing pathways that regulate prospore membrane morphology in meiosis II

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Upon starvation, *Saccharomyces cerevisiae* undergo gametogenesis to form four haploid spores from a precursor diploid cell. During meiosis II, the spindle pole bodies are modified to create a platform for *de novo* synthesis of membranes that grow to encapsulate each of the haploid nuclei, called prospore membranes. During meiosis II, closure of the prospore membrane and disassembly of the meiotic spindle are regulated by the STE20-family GCKIII kinase Sps1, which acts in a pathway with the Hippo-like kinase Cdc15. *SPS1* is required for a meiosis specific phosphorylation of H4S1; this phenotype is shared by a subunit of the TRAPPIII complex, *TRS85*. Because *SPS1* has been previously shown to regulate timely prospore membrane closure, we examined prospore membrane development in *trs85*Δ cells and find that *TRS85* is required for timely meiotic progression and proper prospore membrane morphology. Specifically, *trs85*Δ mutants produce wider prospore membranes compared to wild type cells, a similar phenotype previously described in *sma2*Δ cells. Interestingly, we find that *SMA2* is not required for H4S1 phosphorylation. *SMA2* may act in parallel with *TRS85* for prospore membrane development, as the *sma2*Δ *trs85*Δ double mutants have a more severe prospore membrane morphology phenotype compared to either single mutant. Our results suggest a novel role for *TRS85*, acting in parallel to *SMA2*, to regulate prospore membrane morphology.

202A

The yeast mitochondrial succinylome: Implications for regulation of mitochondrial nucleoids

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Acylations, such as the succinylation of lysine, are post-translational modifications and a powerful means of regulating protein activity. Lysine succinylation occurs predominantly non-enzymatically (with succinyl-CoA as a donor of a succinyl group) and has a profound effect on the corresponding site within the protein, as it reverses the charge of the residue. Mitochondria are the predominant sites of succinyl-CoA production, which arises from the decarboxylation of α-ketoglutarate during the tricarboxylic acid (TCA) cycle; it is therefore expected that mitochondrial proteins are highly prone to succinylation. Although numerous succinylated mitochondrial proteins have been identified in *Saccharomyces cerevisiae*, a more detailed characterization of the yeast mitochondrial succinylome was still lacking. Here, we performed a proteomic MS analysis of purified yeast mitochondria and detected 314 succinylated mitochondrial proteins with 1763 novel succinylation sites. The mitochondrial nucleoid, a complex of mitochondrial DNA and mitochondrial proteins, is one of the structures whose majority of the protein components are affected by succinylation. We found that Abf2p, the principal component of mitochondrial nucleoids responsible for compacting mitochondrial DNA in *S. cerevisiae*, can be succinylated *in vivo* on at least thirteen lysine residues. Abf2p succinylation *in vitro*inhibits its DNA-binding activity and reduces its sensitivity to digestion by the ATP-dependent *Sc*Lon protease. We observed dramatic changes in both number per cell and appearance of mt-nucleoids in cells taken from fermentative, diauxic shift and respiratory phases of the growth. We conclude that changes in the metabolic state of a cell resulting in an increase in the concentration of TCA intermediates may affect mitochondrial functions.

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203A – number intentionally not assigned.

204A

Regulated Secretion Mediates Yeast Cell Fusion

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In yeast mating, partner cells must first degrade the intervening cell wall material before plasma membrane fusion can be achieved. Cell wall removal is carried out by secreted remodeling enzymes such as glucanases, mannanases and chitinases. The action of these enzymes must be tightly controlled, as ectopic cell wall degradation causes cell lysis. Yeast cell fusion requires continuous secretion, as revealed by mutations in the standard secretory pathway. However, electron microscopy demonstrated the appearance of mating-specific vesicles in pheromone-responding cells and at the Zone of Cell Fusion (ZCF) in prezygotes. The vesicles dissipate after fusion is complete. The behavior of the vesicles appears to be regulated by the fusion proteins Fus1p, Fus2p, Rvs161p, and Cdc42p. Deletion of *FUS1* prevents vesicles from localizing to the ZCF; mutations of *FUS2, RVS161 or CDC42* appear to block fusion with the plasma membrane. All lead to defects in subsequent cell wall degradation, but do not show evidence of defects in general secretion. To study the possible role of a mating-specific regulated secretion pathway in yeast, we have developed a Fluorogen Activated Protein (FAP) assay to monitor secretion. The FAP assay works by fusing the FAP to a protein of interest and exposing the cell to a membrane-impermeable fluorogen. When the FAP binds the fluorogen, a fluorescent signal is emitted, allowing for visualization of protein secretion. We FAP-tagged Scw4p and Scw10p, two pheromone-regulated glucanases previously implicated in mating, and monitored their secretion. Scw10p is induced during mating but is secreted in a Fus2p-independent manner. Scw4p expression is shut off during mating, but the secretion of previously synthesized Scw4p at the ZCF is strongly dependent on Fus2p. We conclude that Scw4p is preloaded into the mating-specific vesicles and specifically secreted during conjugation to help effect cell wall removal.

205A

Cellular protein quality control is sensitive to levels of Caj1; a class II J-domain protein in budding yeast

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Protein quality control is an essential process regulating proteins' folding, stability, and turnover. It maintains the cellular protein homeostasis under physiological as well as stress conditions. Hsp70 along with Hsp40s (also known as J-domain proteins or JDPs), play a central role in maintaining protein quality control. Caj1 is one of the JDPs localized in the yeast nucleo-cytoplasmic compartment. Over-expression of Caj1 results into pleiotropic phenotypes, such as filamentous growth, plasma membrane damage and high-temperature sensitivity. We show that over-expression of Caj1 stabilizes a broad range of protein substrates, including plasma membrane, cytosolic as well as non-native proteins in budding yeast. Interestingly, the extent of stabilization varies for different substrates. Accumulation of these misfolded proteins can overwhelm the cellular protein quality control machinery and result in to Caj1 overexpression mediated toxicity. Further, we found that co-overexpression of Ydj1 or Sis1, the two major nucleo-cytoplasmic JDPs rescued Caj1 overexpression mediated defects. Based on our results, we propose that higher levels of Caj1 sharing the same sub-cellular compartment may engage in complementary or competing interactions with other JDPs, their clients, or interactors, thus causing a protein quality control defect.

206A

Re-evaluating the glucose-dependent nuclear localization of hexokinase 2

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Glucose is a critical energy source for cells and serves as a building block for key biosynthetic processes. Hexokinases are central regulators of glucose metabolism, facilitating the first committed step in glycolysis: conversion of glucose to glucose-6-phosphate. Hexokinases are conserved across eukaryotes, and therefore we use *S. cerevisiae* as a model to study hexokinase function. Yeast express three hexokinases with hexokinase 2 (Hxk2) as the predominant isoform in glucose-grown cells. In addition to phosphorylating glucose, Hxk2 has been proposed to regulate the glucose repression pathway by translocating into the nucleus to regulate transcription of metabolic genes with the transcriptional repressor Mig1. Nuclear shuttling of Hxk2 is regulated by phosphorylation, and this modification also controls the dimer to monomer transition for Hxk2, making these two states intimately linked. Using advanced confocal microscopy and automated image quantification approaches, we refine the model of Hxk2 nuclear shuttling, refuting the long-held view that Hxk2 translocates into the nucleus in high-glucose conditions. We instead present evidence that Hxk2 shuttles to the nucleus when glucose is depleted, a response observed in mammalian hexokinases. We expand our understanding of cytosolic-nuclear shuttling for Hxk2 by identifying Tda1, a kinase known to control Hxk2 multimerization in response to glucose depletion, as key for controlling Hxk2 nuclear localization. We show that phosphorylation of Hxk2 at residue serine 15, once thought to be critical for regulating nuclear shuttling, is dispensable for Hxk2 localization changes, but does control monomer-dimer transitions. We further define the role of Hxk2 regulatory sequences in controlling nuclear translocation and define the transcriptional impact of Hxk2 on cells. In marked contrast to earlier studies, we find that Hxk2 plays a very minor role in transcript regulation in glucose grown cells, with only a few genes changing their expression profiles. Taken together, our studies provide insight into the critical roles hexokinases play in regulating fundamental metabolic responses in cells.

207A

A structural and functional analysis of *Nematostella vectensis* major intrinsic proteins in *Saccharomyces cerevisiae*

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There is currently a major gap in understanding tissue grade organisms from early diverging phyla, particularly within the context of understanding placatory responses to osmotic variabilities that can result from salinity fluctuation due to global climate change. A key protein family essential for the regulation of intracellular water and solute concentrations are the Major Intrinsic Proteins (MIPs), these include aquaporins (Aqps) and aquaglyceroporins (Glps). Here, we report the identification and functional analysis of MIPs in *Nematostella vectensis*, a member of the early diverging phyla that represents a unique group of tissue grade organisms. Using a molecular evolution approach, we successfully identified each MIP node in *N. vectensis*. Putative MIPs were cloned and recombinantly expressed in budding yeast, *Saccharomyces cerevisiae* and tested for functionality. *N. vectensis* MIPs displayed varying degrees of functionality, ranging from no effect to lethality, suggesting a phylogenetic approach paired with an *in vivo* functionality assay is critical for their characterization. Total *N. vectensis* MIP transcripts were also quantified during acute and long-term salinity exposure which also support our functional yeast results. Furthermore, based on high confidence protein structure homology modeling, we demonstrate a significant correlation between biophysical pore properties that can accurately predict functionality.

208A

Fission yeast polycystin Pkd2 antagonizes the Hippo pathway Septation Initiation Network in cytokinesis

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Polycystins are conserved eukaryotic calcium channel. Mutations of the human polycystins lead to a common genetic disorder, Autosomal Dominant Polycystic Kidney Disorder (ADPKD). This renal disease results in excessive kidney cell proliferation leading to renal failure

however the underlying mechanism remain unclear. The fission yeast possesses a single essential homologue—Pkd2 that has distinct role in cytokinesis. Here, we examined how Pkd2 interacts with the essential signaling pathways of cytokinesis. We screened for genetic interaction of Pkd2 with more than thirty cytokinesis gene mutants. We identified epistatic genetic interactions between *pkd2* and Septation Initiation Network (SIN), the essential fission yeast Hippo pathway required for cytokinesis. Depletion of Pkd2 partially rescued the viability of a majority of temperature-sensitive SIN mutants. Pkd2 knock down mutant (*pkd2-81KD*) restored septation in most SIN mutants and prevented them from lysis. To understand the underlying mechanism, we isolated a novel temperature-sensitive mutant *pkd2-B42* which is inviable at 36°C. Rod-shaped fission yeast cells grow by tip expansion, the rate of which decreased by 80% in *pkd2-B42*. The *pkd2-B42* cells frequently shrank while losing ~30% of their volume temporarily. Turgor pressure of *pkd2-B42* was 50% of the wildtype. All these phenotypes indicated Pkd2s essential role in maintaining osmolarity. *pkd2-B42* exhibited negative genetic interactions with the calcium signaling mutants of either calmodulin (*cam1-E4, cam1-E14*) or calcineurin (*ppb1D*). However, both the calcineurin(*cam1-E14*) and calmodulin (*ppb1D)* mutant showed negative genetic interaction with SIN mutants. We conclude that the fission yeast polycystin Pkd2p regulates the intracellular osmolarity and antagonizes the SIN pathway in cytokinesis.

209A

RNA editing by dysregulated Adenosine Deaminase Acting on RNA (ADAR) enzyme induces proteotoxic stress

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Traditionally, DNA mutations are considered to bear the sole responsibility for alterations in genomic information. However, A-to-I RNA editing mediated by the RNA editing by the adenosine deaminase acting on RNA (ADAR) enzyme, in which genomically encoded adenosines are transformed and recognized as guanosines in the RNA sequence, is an endogenous and powerful means of creating inner transcriptome diversity. The magnitude of A-to-I RNA editing is unprecedented, with millions of sites already identified in the human genome. When editing occurs within mRNAs, it can recode specific codons, leading to changes in protein structure and function. In mammals, the vast majority of these editing events are usually located within the noncoding Alu elements, and thus these enzymes only

rarely recode proteins. However, we recently found that cephalopods are an intriguing exception: the majority of their mRNAs harbor recording events, and this creates enormous protein diversity. Because cephalopods are behaviorally complex, these findings raise the possibility that massive transcriptome diversification enables phenotypic diversity and flexibility. An interesting prediction is that as a cost, similarly to DNA mutations, it can also serve as a source for proteotoxic stress. To test this possibility, we exogenously expressed ADAR proteins in our favorite model system, the yeast Saccharomyces cerevisiae, an organism whose origins precede the emergence of ADAR, but can express ADARs originated from different organisms. The results showed that such expression caused massive RNA editing, which significantly reduced cell fitness. Furthermore, mass-spectrometry analysis revealed that many of the RNA editing events resulted in increased protein diversity, which enhanced their ubiquitination and tendency to aggregate. Hence, our results suggest that the A-to-I RNA editing events induced by ADAR dysregulation are manifested at the proteomic levels and are an overlooked source of proteotoxic stress.

210A

The sporulation-specific MAPK Smk1 plays a role in prospore membrane development late in meiosis II

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In *S. cerevisiae*, during meiosis II, each haploid nucleus is surrounded by a prospore membrane, which is synthesized *de novo*. The leading edge protein complex (LEP) assembles at the open end of growing prospore membranes and is thought to keep elongating prospore membranes open as meiosis II progresses. *SMK1* is a sporulation-specific Mitogen Activated Protein Kinase (MAPK) that is essential for spore development in *S. cerevisiae*. *SMK1* encodes a non-canonical MAPK, activated by a complex mechanism which includes autophosphorylation on its activation loop in response to the binding of Ssp2. We see that Smk1 exhibits a dynamic localization pattern; it is initially localized along prospore membranes and then localizes specifically to the leading edge of the growing prospore membrane around the time of prospore membrane closure. We see that this dynamic localization of Smk1 requires two components of the leading edge, *ADY3* and *SSP1*, as well as the Smk1 activator, *SSP2*. We find that *SMK1* plays a role in prospore membrane development, as loss of *SMK1* leads to the generation of anucleate membrane compartments late in prospore membrane development. Appropriate late development of the prospore membrane requires Smk1 kinase activity: cells lacking the Ssp2 activator also leads to the formation of anucleate membrane compartments in meiosis II and mutations in *SMK1* that affect its kinase activity show the same prospore membrane phenotypes as the null allele. Our results suggest that *SMK1* plays a role late in prospore membrane development in addition to its previously described role in spore wall deposition.

211A

Dry3, a novel yeast hydrophilin is required for desiccation tolerance

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Most of our understanding of biology occurs within a narrow moisture window. Macromolecules, cells, and organisms typically require above 98% relative humidity to function. Yet, survival at an extremely low water level (termed anhydrobiosis), is essential for most seeds, spores, and microscopic animals. How macromolecules, cells, and organisms can establish and maintain reversible suspension of biological activity at low water content is not well understood. To elucidate how life can persist without water, we must understand how molecules, cells, and organisms protect themselves during desiccation and rehydration. Research in the Tapia lab is centered on understanding how the yeast *Saccharomyces cerevisiae* becomes desiccation tolerant. Previous findings from our lab have demonstrated that yeast can become

desiccation tolerant upon nutrient depletion or through the introduction of specific protectants (trehalose and specific hydrophilins). Hydrophilins are a group of proteins that have been shown to play an important role in desiccation tolerance of a variety of anhydrobiotes and are characterized by their small size, positive charge, and their intrinsically disordered structures. We have previously shown that Hsp12, the most highly expressed yeast hydrophilin, plays an important role in yeast desiccation tolerance likely by modulation damaged membranes. Recent findings demonstrate that the absence of an uncharacterized yeast hydrophilin, which we will refer to as Dry3 (***D***esiccation ***R***esistance in ***Y***east ***3***), leads to a significant decrease in desiccation tolerance, especially with extended periods of drying. Dry3-GFP demonstrates membrane localization into eisosome-like compartments. We are currently investigating the relationship between Dry3 and eisosomes as it relates to desiccation and how a this simple hydrophilin is mediating desiccation tolerance.

212B

Regulation and consequences of SNF1/AMPK complex control across different stress conditions

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The capacity to adapt accordingly to a changing environment is crucial for organismal survival. The SNF1/AMPK protein kinase family is widely conserved in mammals, fungi, and plants, and in yeast, it represents a master regulator of cell adaptation. SNF1/AMPK plays roles in cell response to environmental stress, including carbon and oxidative stress; as well as in other important cellular processes and signalling pathways. The SNF1 complex is formed by three subunits, the Snf1 catalytic subunit, Snf4 gamma subunit and one of the three beta subunits, Gal83, Sip1 and Sip2. The mechanism of SNF1 complex activation following carbon stress has been widely studied where it functions as a master regulator of transcription enabling glucose-dependent repression/derepression of gene expression that is central to the yeast Crabtree effect. However, relatively little is known in terms of the role of SNF1/AMPK in other stress responses. In this study, we have addressed the SNF1/AMPK complex integrity, and the role impact of three upstream regulators Sak1, Tos3 and Elm1 in the response to both glucose depletion and oxidative stress. We have also addressed the role of Snf1 and its regulation across the cell cycle under these stresses. Our studies highlight stress specific co-ordination of SNF1/AMPK function allowing a complex network of signalling interactions which align the adaptive response to the stress imposed.

213B

Coq11 is an atypical short-chain dehydrogenase/reductase (SDR) that modulates coenzyme Q (CoQ) biosynthesis

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Coenzyme Q (CoQ or ubiquinone) is a redox-active lipid molecule that acts as an electron carrier in the mitochondrial electron transport chain, aiding in the mitochondrial production of ATP. In *Saccharomyces cerevisiae*, at least 14 nuclear encoded proteins are required for efficient mitochondrial biosynthesis of CoQ_6, an isoform of CoQ with a hexaprenyl “tail” of six isoprene units. Many of the Coq polypeptides (Coq3-Coq9 and Coq11) localize to the matrix side of the inner mitochondrial membrane where they assemble into a high molecular mass complex known as the CoQ Synthome. The correct assembly of this complex is required for efficient CoQ biosynthesis, as it is destabilized by individual deletion of *COQ* genes, resulting in severe defects in CoQ biosynthesis. Recently, the polypeptide Coq11 was identified as a novel member of the CoQ Synthome. Deletion of *COQ11* has been shown to significantly reduce, but not abolish, *de novo* CoQ_6 biosynthesis. Despite its impaired CoQ_6 biosynthesis, the *coq11*Δ mutant appears to display a putatively more stable or enlarged CoQ Synthome and retains the antioxidant properties afforded by ubiquinol ($CoQH_2$). While several roles for Coq11 have been hypothesized, its function in CoQ_6 biosynthesis has not yet been fully elucidated. Sequence analyses have identified Coq11 as a member of the short-chain dehydrogenase/reductase (SDR) superfamily, specifically as a member of subgroup five of the atypical SDRs. The SDRs constitute a large family of catalytically diverse enzymes that, despite having low pairwise sequence identities, share a relatively conserved catalytic triad or tetrad, several loosely conserved sequence motifs, and a conserved N-terminal Rossmann-fold, a protein structural motif utilized in the binding of a dinucleotide cofactor. This work aims to characterize the function of Coq11 using its identity as a member of the SDR superfamily. CRISPR-Cas9 mediated genome editing was used to introduce point mutations in the yeast genome at residues that comprise the hypothesized Coq11 SDR catalytic tetrad. Strains harboring these point mutations were phenotypically characterized by examining respiratory capacity, *de novo* CoQ_6 biosynthesis, and retention of the antioxidant protection afforded by ubiquinol. Overall, the results of this work provide insight into the function of an atypical SDR required for efficient CoQ_6 biosynthesis in yeast.

214B

Integrating multiple single-cell phenotypes in the yeast stress response links activity of the ribosome-gene transcriptional repressor Dot6 to faster acclimation after stress

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Stress defense and cell growth are inversely related in bulk culture analyses, such that cells often show slower growth after exposure to an environmental stress. However, bulk culture studies fail to capture cellular heterogeneity, which can produce unique phenotypic relationships found only in subsets of cells. To investigate this heterogeneity, we paired live-cell imaging with a microfluidics system to study multiple phenotypes of single *Saccharomyces cerevisiae* cells before and after salt stress. We simultaneously followed cell and colony growth, cell size and volume, cell-cycle phase, and nuclear localization of two co-regulated stress responsive transcription factors, transcriptional activator Msn2 that regulates stress-induced genes and repressor Dot6 that represses expression of ribosome-biogenesis genes. We found unexpected differences in Msn2 and Dot6 localization changes across cells, revealing unanticipated discordance in their regulation.

Although past work connected Msn2 activation to growth rate, we instead found strong correlations with Dot6 behavior: cells with a larger Dot6 nuclear relocalization response showed faster acclimation of growth rate after stress, whereas cells with a weaker Dot6 response showed slower acclimation. This was surprising, since Dot6 is predicted to repress the expression of growth-promoting genes. Indeed, cells lacking *DOT6* and its paralog *TOD6* show normal growth rate before stress, but show delayed acclimation to salt stress, indicating that these repressors are part of the adaptive stress response. We found that post-stress growth rate could be partly predicted by integrating multiple cellular phenotypes, wherein the magnitude of Dot6 nuclear translocation had among the highest predictive power of post-stress growth rate. Our results underscore that life-history experience partially predicts how cells will response to stress.

215B

The regulation of endoplasmic reticulum stress in *Candida albicans*

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Candida albicans is the leading cause of yeast infections in humans. Despite the availability of antifungal drugs to treat *C. albicans* infections, mortality rates amongst immunocompromised individuals remain high, and *C. albicans* resistance to antifungal drugs is becoming more frequent. A cellular program termed the Unfolded Protein Response (**UPR**), which functions to alleviate the toxic accumulation of misfolded proteins in the endoplasmic reticulum (ER), has been found to play a crucial role in *C. albicans* resistance to antifungal drugs and ability to cause disease. In *C. albicans*, the UPR is activated via a single stress-sensing molecule, Ire1. Various small molecules have been shown to attenuate ER stress and improve protein folding in the cell, including the bile acid tauroursodeoxycholic acid (TUDCA). Although the UPR is well characterized in the simple yeast *Saccharomyces cerevisiae*, the specific UPR target genes that regulate *C. albicans* antifungal resistance and pathogenicity are unknown. In agreement with previous studies, we found that repression of *IRE1* sensitizes *C. albicans* to various antifungal drugs and reduces its ability to undergo filamentation. We also showed that administration of TUDCA improved growth of yeast treated with the ER stressor tunicamycin and the antifungal drugs caspofungin and amphotericin B. However, TUDCA significantly exacerbated fluconazole toxicity. Our findings support the role of *IRE1* as an essential component in *C. albicans* antifungal resistance and pathogenicity and furthermore suggest that TUDCA can differentially potentiate the toxicity of antifungal drugs.

216B

Respiratory defects caused by mutations affecting the Endoplasmic Reticulum-Mitochondria Encounter Structure (ERMES) can be rescued by deletion of *COQ11*

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Coenzyme Q (CoQ) is an essential redox-active lipid that plays a major role in the electron transport chain, driving mitochondrial ATP synthesis. Deficiency of CoQ causes a wide range of clinical deficiencies, highlighting the need to study the biosynthesis of this lipid to design therapeutics to treat these symptoms. In *Saccharomyces cerevisiae*, CoQ biosynthesis takes place exclusively in the mitochondrial matrix using a mega complex, the CoQ Synthome, which is comprised of Coq3-Coq9 and Coq11. A recently identified regulator of CoQ Synthome assembly and CoQ production is the ER-mitochondria encounter structure (ERMES). ERMES is a tethering complex that bridges the two organelles, and the CoQ Synthome resides in specific membrane niches directly adjacent to this complex. Loss of ERMES results in transcriptionally upregulated expression of *COQ* genes, yet inefficient synthesis due to a destabilized CoQ Synthome. In this work, *ERMESΔcoq11Δ* mutants have been generated in an effort to correct this defect, as deletion of *COQ11* has been shown to promote mitochondrial CoQ content and enhance CoQ Synthome stability, subsequently rescuing the respiratory deficient *coq10Δ* mutant. We seek to investigate the relationship between the ERMES complex and the CoQ biosynthetic complex to better understand the regulation of CoQ biosynthesis and aid in the development of more effective therapeutics for diseases linked to CoQ deficiencies.

217B

Amino acid mimicry: Insights into glyphosate transport and mitochondrial toxicity

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Glyphosate, the most widely used pesticide in the world, directly inhibits the shikimate pathway. Even though humans lack this pathway, a number of reports have indicated various toxic effects, suggesting that glyphosate targets other pathways too. In *Saccharomyces cerevisiae*, deletion of Dip5, a membrane glutamate/aspartate transporter, confers resistance to glyphosate, suggesting that despite its classification as a glycine analogue it resembles the size and charge distribution of glutamate and enters the cell through Dip5. Additionally, previous work from our group demonstrated that glyphosate changes expression of mitochondrial associated genes. Therefore, we tested if mitochondrial glutamate transporters are able to import glyphosate by determining if their deletion confers resistance, similar to *dip5* knockouts. Here we show that loss of two mitochondrial glutamate transporters in *S. cerevisiae* confers resistance to glyphosate. We further establish a dose equivalence between pure glyphosate and a commercial formulation, indicating that commercial formulations are more potent, perhaps due to increasing permeability through surfactants. We then examine the effects on nutrient availability on glyphosate and commercial formulation toxicity. This further corroborates the hypothesis that the structural similarity of glyphosate to glutamate allows it to utilize glutamate permeases to enter the mitochondria. This evidence also indicates that glyphosate likely has mitochondrial off-targets, potentially suggesting mechanisms for toxicity in organisms that rely on their diets for aromatic amino acid intake. We anticipate our results to be a starting point for more in-depth analysis of the effects of glyphosate on mitochondrial function, as well as further work on confirming the mechanisms of intracellular and intramitochondrial glyphosate transport.

218B

Spo77 acts in a pathway with Cdc15 and Sps1 to regulate exit from meiosis II

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Exit from meiosis II in *S. cerevisiae* involves several cellular events: meiotic spindles made of microtubules are disassembled after nuclear division, the phosphatase Cdc14 is released from the nucleolus, and prospore membranes grow around and encapsulate each of the four nascent nuclei to form four spores within the ascus. *CDC15*, a Hippo-like kinase and *SPS1*, a STE20-family GCKIII kinase, are required for these meiosis II exit events to occur appropriately. *SPO77* was identified as a high copy suppressor of an *sps1* hypomorphic allele. We demonstrate that Cdc15, Spo77, and Sps1 form a complex in meiosis II. We show that *SPO77* plays a role in meiosis II exit and is required for the sustained release of Cdc14, in addition to its previously identified role in timely prospore membrane closure. We also see that *CDC15* is required for the post-translational modification of Spo77 while *SPS1* is not required for this modification. These results suggest that *SPO77* acts in a pathway downstream of *CDC15* with *SPS1* to regulate exit from meiosis II.

219B

Elucidating the uptake of exogenous coenzyme Q and its delivery to mitochondrial respiratory complexes

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Coenzyme Q (CoQ) is a redox-active lipid synthesized within the mitochondria and is the third most consumed nutritional supplement after fish oil and multivitamins. CoQ plays essential roles in antioxidant protection and mitochondrial bioenergetics. Aging, certain pharmaceuticals, and genetic mutations result in deficiencies in CoQ and disease. However, clinical studies investigating the efficacy of CoQ supplementation benefit just a subset of patients, or often lead to modest or inconsistent results, likely due to the hydrophobicity of CoQ and the several membrane barriers it must cross to assimilate into the mitochondria. More work to understand the pathways of CoQ uptake and trafficking is needed. Our lab has previously identified six genes—*VPS1*, *CDC10*, *NAT3*, *RTS1*, *RVS161*, and *RVS167*—whose protein products represent essential steps in the uptake and trafficking of CoQ in *Saccharomyces cerevisiae.* The genes were identified in *ORFΔcoq2Δ* double knockouts, in which *coq2Δ* abolishes endogenous CoQ biosynthesis and *ORFΔ* removes the uptake and trafficking gene of interest. Total content of exogenous CoQ taken up by *ORFΔcoq2Δ* double knockouts was quantified using liquid-chromatography mass spectrometry (LCMS). Four of the six strains showed a decreased CoQ content, indicating that CoQ uptake may be impaired. *vps1Δcoq2Δ* had a comparable CoQ content to controls, suggesting that CoQ intracellular distribution, but not CoQ uptake, was impaired. Interestingly, *rvs167Δcoq2Δ* showed an increased CoQ content relative to controls. One identified gene of interest, *VPS1*, is involved in membrane fusion and fission in many cellular processes, including both endocytosis and endomembrane trafficking. Transformation of *vps1Δcoq2Δ* with partially functional *vps1* point mutants revealed that the endomembrane trafficking function of Vps1 is essential for CoQ uptake and trafficking, while the endocytic function is dispensable. Next steps will include subcellular fractionation to determine the role of the *ORF* gene in endomembrane trafficking and CoQ transport. Current work uses yeast integrative vectors to re-insert the *ORF* gene into the *HIS3* genomic locus of *ORFΔcoq2Δ* double knockouts to determine if rescue by exogenous CoQ and growth can be more robustly restored without concerns of plasmid maintenance. Results obtained from these studies will shed light on how CoQ, an essential and insoluble lipid, is taken up by cells and trafficked to and from the mitochondria.

220B

Elucidating the identity and function of mitochondrial membrane contact site tethers in budding yeast gametogenesis

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Mitochondria are essential organelles containing their own genome. As such, mitochondrial segregation is a crucial aspect of gametogenesis necessary to produce viable gametes. However, how mitochondria are inherited into gametes remain unknown. Budding yeast gametogenesis provides a powerful model to uncover and dissect mechanisms of mitochondrial inheritance. During budding yeast gametogenesis, the mitochondrial network detaches from the cell periphery upon regulated degradation of a mitochondrial-plasma membrane tethering complex (MECA) and forms extensive membrane contacts with the nuclear envelope in meiosis II as chromosomes are segregated into newly forming gametes. Subsequently, portions of the network are pinched off and re-establish mitochondrial-plasma membrane associations within the nascent gametes. I developed a proximity-dependent labeling strategy to biochemically identify mitochondrial-nuclear envelope tethering factor(s) and investigate their contribution to mitochondrial dynamics and segregation in meiosis. In parallel, I examined the role of previously known and recently discovered mitochondrial tethers, including ERMES, Ltc1, and Cnm1, during gametogenesis. These analyses revealed the individual contributions of these tethers to be largely dispensable for both mito-nuclear contacts and mitochondrial inheritance, suggesting the existence of redundancy between known pathways and/or novel regulators. Finally, I found that ectopic tethering of mitochondria to the cell periphery with an engineered tether increased the production of respiratory-incompetent gametes, suggesting remodeling of mitochondrial contact sites is critical to ensure gamete health.

221B

Meiotic Nuclear Pore Complex Remodeling Provides Key Insights into Nuclear Basket Organization

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Nuclear pore complexes (NPCs) are large proteinaceous assemblies that mediate nuclear compartmentalization. NPCs undergo large-scale structural rearrangements during mitosis in metazoans and some fungi. However, our understanding of NPC remodeling beyond mitosis remains limited. Using time-lapse fluorescence microscopy, we discovered that NPCs undergo two mechanistically-separable remodeling events during budding yeast meiosis whereby parts or all of the nuclear basket transiently dissociate from the NPC core during meiosis I and II, respectively. Meiosis I detachment, observed for Nup60 and Nup2, is driven by Polo kinase-mediated phosphorylation of Nup60 at its interface with the Y-complex. Subsequent reattachment of Nup60-Nup2 to the NPC core is mediated by a lipid-binding amphipathic helix in Nup60. Preventing Nup60-Nup2 reattachment causes misorganization of the entire nuclear basket in gametes. Strikingly, meiotic nuclear basket remodeling also occurs in the distantly related fission yeast, *Schizosaccharomyces pombe*. Our study reveals a conserved and developmentally programmed aspect of NPC plasticity, providing key mechanistic insights into nuclear basket organization.

222B

DNA damage induced SUMOylation regulates nuclear protein quality control in *Saccharomyces cerevisiae*

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Proteostasis is achieved through an intricate network of protein quality control (PQC) circuits that aim to refold, degrade, or sequester misfolded proteins. Recent studies have unveiled a new PQC circuit, wherein upon genotoxic stress, numerous proteins get sequestered to a quality control site in the nucleus called the IntraNuclear Quality (INQ) control. Here, we use budding yeast as our model system to establish Rpd3, a histone deacetylase, as an INQ marker and study the signals that lead to its sequestration. We find that DNA damage induces SUMOylation of two small-heat shock proteins Btn2 and Hsp42 important for INQ localization. We map the SUMO site to the C-terminus of Btn2 and implicate polySUMOylation as a signal for clearance of INQ in the nucleus. We further try to elucidate the relationship between SUMOylation of Btn2 and two protein degradation pathways with respect to INQ clearance. This work identifies a novel chaperone post-translational modification that regulates one of the most poorly understood quality control sites in yeast. Since SUMOylation cascades are such an important part of DNA repair reactions, we think this represents a link in the signaling following genotoxic stress that coordinates protein quality control and genome maintenance activities.

223B

Phosphoregulation of the yeast Pma1 H^+-ATPase autoinhibitory domain is mediated by Ptk1/2 kinases and Glc7 PP1 phosphatase and is under TORC1 control

Nadia Pia Guarini, Elie Saliba, Bruno André ULB

Pma1, the yeast plasma membrane (PM) H^+-ATPase, establishes the H^+ gradient that drives active nutrient uptake and provides the main component of the PM potential. Pma1 also importantly contributes to control of intracellular pH, which is reported to provide a signal for control of cell growth (1). In a previous study, we showed that Pma1 promotes initial reactivation of the TORC1 (Target of Rapamycin Complex 1) kinase complex upon H^+-coupled uptake of replenishing nutrients into starved cells (2). Plant PM H^+-ATPases, which are closely similar to yeast Pma1, also contribute to signaling to TORC1 (3).
The activity of fungal and plant H^+-ATPases is tightly regulated by environmental cues. This control is mediated by their cytosolic autoinhibitory C-terminal tail, the effect of which is modulated by phosphorylation (4). In yeast Pma1, phosphorylation of the C-terminal S911 and T912 adjacent residues plays a key role in regulation by glucose of the H^+ pump's activity (4). However, the kinases and phosphatases controlling this modification remain unknown. We have now found that phosphorylation of S911 and T912 in Pma1 is mediated by the largely redundant Ptk1 and Ptk2 kinases and counteracted by the Glc7 PP1-type phosphatase. Furthermore, Pma1-dependent TORC1 activation upon transport-coupled H^+ influx is impaired in the *ptk2* mutant where S911-T912 are not properly phosphorylated. We also observed that S911-T912 phosphorylation increases when TORC1 is inhibited by rapamycin, suggesting the existence of a feedback control via TORC1 of Pma1's activity. Our results shed new light on Pma1 regulation and Pma1-TORC1 relationships.

224B

Mechanisms and functions of Whi5 phosphorylation at the Start transition of the budding yeast cell cycle

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The budding yeast *Saccharomyces cerevisiae* couples growth to division through dilution of Whi5, a transcriptional inhibitor of the Start transition. Whi5 is not synthesized in G1, such that its concentration decreases as cell volume increases. In addition to Whi5 dilution, Start is also driven by the cyclin-CDK complex Cln3-Cdk1, which promotes activation of SBF, a key transcription factor for cell-cycle-dependent gene expression. Two key targets of SBF are the downstream G1 cyclins Cln1 and Cln2 that, in complex with Cdk1, target Whi5 for phosphorylation and inactivation. To determine how Whi5 phosphorylation regulates Start, we investigated the cell cycle dynamics and phosphorylation patterns of different Whi5 phosphorylation site mutants. In G1, we observed a constant hypo-phosphorylation pattern of Whi5 that is independent of all Cdk1 activity, including Cln3-Cdk1 activity. This is similar to previous observations where the retinoblastoma protein Rb, which fulfills a role analogous to Whi5 in human cells, was consistently monophosphorylated until about 2 hours before S phase.

Thus, prior to Start, there is no evidence of a gradual increase in Whi5 phosphorylation. However, after Start, we observed rapid hyperphosphorylation of Whi5 by Cln1/2-Cdk1, which is enabled by Cks1-dependent priming interactions between Whi5 TP phosphorylation sites. Removal of Cdk1 target sites in Whi5 leads to larger cells and reduced *CLN2* expression, suggesting a disruption of the positive feedback loop between Whi5 inactivation and Cln1/2-Cdk1 activity, which plays a role in the irreversibility of the G1/S transition. Our work supports a model where Whi5 sets the timing of Start in a manner independent of cyclin-CDK activity, and semi-processive Cdk1-dependent phosphorylation mechanisms drive the rapid inactivation of Whi5 post-Start.

225B

Cbf1 as a pivotal point in the partitioning of glucose to respiration and lipid biogenesis through differential function of its phosphorylated forms.

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Centromere binding factor 1 (Cbf1) is a global transcription factor that controls the transcription of many genes involved in cellular respiration and lipid biogenesis in yeast. It is a known substrate for PAS kinase, which phosphorylates Cbf1 and alters its activity, inhibiting its respiratory function. Our previous findings suggest that Cbf1 normally functions to activate respiration in yeast, with a Cbf1 knockout displaying decreased respiration. This activity is inhibited by phosphorylation at threonine T211, with a T211A or PAS kinase mutant displaying increased respiration. Cbf1, and its human homolog USF1, are also known to regulate lipids, directly controlling the expression of yeast ceremide synthesis genes. In fact, human alleles of USF1 have been associated with familial hyperlipidemia and cholestrolemia in several studies, presumable due to its known role in the regulation of key genes involved in fatty acid synthesis including Fatty Acid Synthase (FAS). Thus, Cbf1 is a pivotal point in the regulation of glucose partitioning towards either respiration or lipid biogenesis. Both Cbf1 and USF1 are well-known to bind promoters at a conserved sequence, but they are also known to work with other transcription factors in the regulation of their targets. Our hypothesis is that phosphorylation of Cbf1 by PAS kinase affects the activity and hence transcriptional targets of Cbf1 by altering the protein binding partners of Cbf1. In this study, we perform Cbf1 interactome screens to determine what transcription factors Cbf1 interacts with in its phosphorylated versus unphosphorylated state. These results suggest mechanisms by which cells regulate central metabolism, which lies at the heart of most metabolic disease.

226B

Differential regulation of Yeast Osh and ORP genes, supporting the evolution of genes for different functions and pathways

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The sterol and lipid metabolism proteins, oxysterol binding proteins (OSBP) and OSBP-related proteins (ORP) have been implicated in various human diseases including dyslipidemias and amyotrophic lateral sclerosis. These proteins are mammalian homologs of the yeast Osh proteins. It has been suggested that the yeast genes Osh6 and Osh7, which are homologues of human ORP5 and ORP8, have redundant, overlapping function. However, genes do not evolve to have the precisely the same function and pathways. Genes evolve to have some selective advantage no matter how small that may be to be able to persist in the population. The Osh6 and Osh7 proteins appeared to have evolved through gene duplication, and we submit that they have since begun to differentiate. We seek to characterize the differential regulation and function of these related yeast genes.

In our study three major differences were noted. First, these genes are phosphorylated and regulated differently. Snf1 and its human homologue AMPK phosphorylates Osh7/ORP8 directly but not Osh6 as shown through both in vitro kinase assays as well as a yeast-2-hybrid system to detect protein-protein interactions. In addition, using fluorescent microscopy, it was determined that Osh7 but not Osh6 colocalizes to ER-mitochondrial junction using mdm34. The final supporting claim was from tests carried out to determine mitophagy and effects on respiration. These suggested that Osh6 plays a more prominent role in mitophagy and mitochondrial dysfunction, where ΔOsh6 displayed reduced respiration rates compared to ΔOsh7.

To conclude, these assays suggest that indeed Osh6 and Osh7 have differences in regulation and in function, supporting the claim that genes have differentially evolved to perform independent functions.

227B

Adopting an ORFan and *AIM*(*33*)ing for a function.

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In participating in the Yeast ORFan Gene Project, we sought to characterize *AIM33*, a gene with unknown role in molecular function, involvement in biological process, or location in cellular component. Constructing a complete understanding of the *Saccharomyces cerevisiae* genome by coordinating strategies will add to the many reasons that baker's yeast is a great model organism for a variety of research areas relating to mammalian cellular mechanisms. We focused on characterizing the gene *AIM33* using bioinformatics databases and devised specific experimental assays to confirm results obtained via large-scale surveys and to gain more insight to protein function. There is strong evidence that Aim33 is a cytochrome b5 reductase, contains NAD and FAD binding domains, and is involved in metabolic processes and inheritance of mitochondria. Large scale finding that knocking out *AIM33* increased mitochondrial inheritance is consistent with our experimental assay results. Compared to WT cells, more ***aim33Δ*** cells remained respiratory competent in a petite frequency assay suggesting that Aim33 may be involved in regulating mitochondria biogenesis. We further found ***aim33Δ*** is more resistant to ethanol and nefazodone, both compounds interfere with mitochondrial functions; however, growth of ***aim33Δ*** cells were similar to WT when exposed to

oxidizing agent H_2O_2, or to molecules that caused genome instability or disrupted protein synthesis. It is possible that the unregulated mitochondria inheritance phenotype is secondary to the defect in NAD or FAD-dependent metabolic pathways.

228B

TORC1 Signaling Controls the Stability and Function of α-Arrestins Aly1 and Aly2

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Nutrient supply dictates cell signaling changes, which in turn regulate membrane protein trafficking. To better exploit nutrients, cells relocalize membrane transporters via selective protein trafficking. Key in this reshuffling are the α-arrestins, selective protein trafficking adaptors conserved from yeast to man. α-Arrestins bind membrane proteins, controlling the ubiquitination and endocytosis of many transporters. To prevent the spurious removal of membrane proteins, α-arrestin-mediated endocytosis is kept in check through phospho-inhibition. This phospho-regulation is complex, with up to 87 phospho-sites on a single α-arrestin and many kinases/phosphatases targeting α-arrestins. To better define the signaling pathways controlling paralogous α-arrestins, Aly1 and Aly2, we screened the kinase and phosphatase deletion (KinDel) library, which is an array of all non-essential kinase and phosphatase yeast deletion strains, for modifiers of Aly-mediated phenotypes. We identified many Aly regulators, but focused our studies on the TORC1 kinase, a master regulator of nutrient signaling across eukaryotes. We found that TORC1 and its signaling effectors, the Sit4 protein phosphatase and Npr1 kinase, regulate the phosphorylation and stability of Alys. When Sit4 is lost, Alys are hyperphosphorylated and destabilized in an Npr1-dependent manner. These findings add new dimensions to our understanding of TORC1 regulation of α-arrestins and have important ramifications for cellular metabolism.

229B

Cadmium Quantum Dots Affect Receptor Mediated Endocytosis and Cell Polarity

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Quantum dots (QDs) are nano-sized semiconductor crystals that are highly utilized for research and medical applications. Recent findings have indicated the toxicity of QDs, however, its impact on yeast remains unclear. The primary goal of our research was to investigate the interaction between QDs and *Saccharomyces cerevisiae* as well as the induced changes resulted from this interaction. We treated red cadmium selenide zinc sulfide quantum dots (CdSe/ZnS QDs) to yeast strains expressing different organelle reference markers to track QDs' subcellular localization. We found that QDs initially interacted with the outer components of yeast cells with a preference for the mother site. Around 6 hours after treatment, QDs were found at the plasma membrane, the endocytosis vesicle, and the late Golgi/trans Golgi network. QDs was not observed in the late endosome. Additionally, we found that 6 hours of QDs exposure caused various abnormalities, including prolonged turnover rate of endocytosis associated protein, Abp1-GFP; depolarization of GFP-Snc1; and fragmentation of the actin cable. Interestingly, results from the recovery assay showed that actin cable fragmentation resulted from QDs exposure is reversible. Overall, this study provided an insight for yeast-QDs interactions.

230B

Stress-related localization of RNA processing proteins to the mitochondria

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Recent developments identified a number of proteins involved in RNA processing that are predicted to gain mitochondrial targeting signals and localize to mitochondria based on ribosome profiling data and functional assays. We investigated possible mitochondrial localization of the translational repressor Ssd1 and the RNA helicases Dhh1 and Prp43 through fractionation of TAP-tagged strains followed by immunoblotting. Analysis of this fractionation by SDS-PAGE gel electrophoresis and immunoblotting confirms protein presence within the cell, the isolated cytoplasm, and most importantly, in the isolated mitochondria. Initial studies on localization in response to heat and oxidative stress will be presented. We are also investigating the possible functions of the proteins in the mitochondria based on their known functions elsewhere in the cell.

231B

Whi2 beyond TorC1 regulation

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Saccharomyces is well suited to thrive in nitrogen rich environments as well as tolerate leans ones. These abilities derive from a finely tuned regulatory system that responds to the constantly changing environments yeast cells face. Target of Rapamycin Complex 1 (TorC1) and Gcn2 are the central protein kinases of this nitrogen-responsive regulatory system. Their activities are exquisitely regulated by intracellular amino acid concentrations. TorC1 and Gcn2 regulate their downstream targets in opposite ways and cross-regulate each other's activities, thus achieving the buffered responses needed to prevent over-reactions as conditions change. Whi2 is a recent addition to this regulatory pathway (Chen *et al*. 2018). In mutant screens, *whi2* cells were identified along with *npr2* and *npr3* mutants, which contain defective SEACIT components that down-regulate TorC1 by inactivating the RAG-like, TorC1 activating GTPase Gtr1. This observation argued that Whi2 might also down-regulate TorC1, an idea confirmed by the demonstration that Whi2 and its phosphatase binding partners Psr1/Psr2 are

required to suppress TorC1 activity-dependent phosphorylation of Rps6 when cells are shifted from a high to low amino acid-containing medium. Whi2's suppression of TorC1 activity predicted it should also up-regulate the Nitrogen Catabolite Repression (NCR)-sensitive transcriptional activators Gln3 and Gat1. Although initially supported by a Gat1-GFP reporter experiment, more detailed analyses revealed that Whi2 exerts only minor influence over NCR-sensitive transcription and then on the NCR-sensitive genes whose products are associated with the massive amino acid interconversions that occur during amino acid downshifts. What then were the major downstream targets of Whi2 regulation? Our proteomic analyses of wild type and *whi2*Δ cells revealed the levels of 58 proteins changed $\log_2$ <-3 to -8 or >+3 to +8. Most surprising, however, these proteins were associated with only two GO-terms, carbohydrate metabolism and oxidative stress. The bulk of the proteins with highly altered levels in *whi2*Δ cells mediated a broad spectrum of unrelated functions. Conspicuously missing were many expected TorC1-regulated target proteins. These observations argue that Whi2-Psr1/Psr2 complex performs many more functions beyond its impact on TorC1 activity and form the basis for future investigations of these functions. NIH GM35642-27, Van Vleet Chair, LTAUSA18162, RVO 61388971.

232B

Studying the Mechanism for Ricin-Induced Apoptosis Daniel Judge West Virginia University

Apoptosis can be triggered via a wide variety of cellular mechanisms, many of which are not well understood. One such mechanism results from rRNA damage after exposure to the poison ricin. Ricin is a multisubunit, carbohydrate-binding protein found in the seeds of *R. communis*. While the function of the catalytic subunit of ricin, ricin toxin A chain (RTA), is well known, the ultimate process initiating apoptosis in RTA-compromised cells is not. RTA depurinates (cleaves a purine nucleotide) at a conserved rRNA site across multiple species, preventing the necessary exchange of GTP to GDP during the elongation step of translation, stalling ribosomal function, and halting protein synthesis. However, ricin poisoning results in the depurination and inactivation of less than two percent of a yeast cell's ribosomes, not enough to significantly impair translational efficiency. Additionally, while ricin causes apoptosis, other compounds, such as the antibiotic anisomycin, that inhibit ribosomal function through mechanisms other than depurination often only cause cell cycle arrest, suggesting that the mechanism of depurinating ribosomes itself is a key to somehow initiating apoptosis. One theory we believe is that stalled ribosomes in a polysome, multisubunit ribosomal complexes linked by mRNA during translation, can collide with one another, and the resulting physical damage activates ribosome quality control mechanisms leading to apoptosis. The following specific aims are proposed to study RTA-induced apoptosis: 1. Using an *in vivo* ricin reporter to characterize *S. cerevisiae* apoptotic response, 2. Using *S. cerevisiae* genetic screens to identify elements necessary for ricin-induced apoptosis, and 3. Performing in-lab evolutions to identify mutations that resist ricin-induced apoptosis. Synthesis of an *in vivo* ricin reporter in yeast will be used to modulate gene expression of RTA via a tetracycline regulatable promoter. Ribosomal biogenesis, localization, and degradation will be monitored before and during apoptosis. Wild type RTA will then be compared to the catalytically dead E177K mutant and others, such as S215F and P95L, that don't induce apoptosis. Finally, knockout screens will be carried out to determine factors necessary for ricin-induced apoptosis. Long-term, this research will result in a better understanding of the cellular stress pathways affected after exposure to ricin and a controlled method for reliably inducing apoptosis in targeted cells.

233V

Strain dependent insulation of the HOG and mating pathways Taylor D Scott[1], Ping Xu[2], Althys Cao[1], Megan McClean[1,21] Biomedical Engineering, University of Wisconsin-Madison, [2]Lewis-Sigler, Princeton University

Cells respond to extracellular signals via tightly regulated series of molecular reactions known as signaling networks. Signaling networks are connected and reactions may occur in several signaling networks. The high osmolarity glycerol (HOG) pathway controls the yeast response to osmotic stress and shares reactions with the mating and filamentous growth (FG) pathways, which control the response to pheromone and starvation respectively. Despite this, the pathways are isolated, and signal does not flow from the HOG pathway into the mating/FG pathways via this connection, in part due to the strong inhibitory effects of the HOG pathway MAPK Hog1p on mating/FG pathway activation. A consequence of this insulation is that cells exposed to simultaneous osmostress and pheromone cannot fully activate the mating pathway until they have adapted to the osmostress. Thus, osmostress dampens and delays mating pathway activation.
We have used integrated fluorescent reporters of HOG and mating/FG pathway activity to quantitatively measure the response to simultaneous osmostress and pheromone in two strain backgrounds. The known structure of the HOG and mating/FG MAPK pathways in these strains is identical. We found that both strains exhibit identical dampening at every level of osmostress. However, in one strain (congenic with S288C), the delay is constant at approximately 45 minutes at every concentration of sorbitol. In contrast, the delay in the second strain is shorter and dose-dependent, ranging from 10 minutes at low osmostress to 40 minutes at high osmostress. We find that in the second strain, but not the first, mating/FG pathway transcripts are induced following an osmotic shock in the absence of pheromone. Experiments in mating/FG pathway mutants show that this induction is consistent with signal leaking from the HOG pathway into the mating/FG pathways, also known as crosstalk, despite the insulation between these pathways. We investigated the effects of Hog1p activity on crosstalk in the two strains and found that Hog1p activity is necessary to suppress crosstalk late in an osmotic shock time course but is not critical for suppressing the early crosstalk seen in the second strain. This suggests that a second, Hog1p independent mechanism, suppresses early crosstalk in the first, more standard strain. We explore the physiological consequences of crosstalk to learn how pathway insulation may affect the ability of cells to correctly respond to multiple signals.

234V

Analysis of cell elongation phenomena caused by Massive expression of EGFP shotaro namba[1], Hisao Moriya[21] Environmental and Life science, Okayama University, [2]Okayama University

Overexpression of proteins sometimes has a negative effect on cellular function. One of the effects of protein overexpression has been discussed in terms of protein burden/cost, which is understood as the growth inhibition observed when overexpressing non-harmful proteins

that have no physiological function in the cell. Many of the details of protein burden are unknown, such as which of the protein-producing resources are overloaded to cause growth inhibition. Fluorescent proteins (EGFP, Venus, mCherry) have been used as model proteins for non-harmful proteins. This is because fluorescent proteins have no physiological function in cell and do not interact with other proteins.

In this study, we analyzed the phenomenon of growth inhibition caused by overexpression of EGFP. Our results revealed that EGFP is a harmful protein that overloads processes other than protein production resources. First, we found that overexpression of EGFP causes cell elongation in yeast. Further analysis with several fluorescent proteins and their mutants revealed that the cysteine content of the proteins is associated with cell elongation. Particularly pronounced cell elongation was observed when EGFP was overproduced in the proteasome mutants. Furthermore, the formation of protein aggregates and the transcriptome changes associated with overexpression of EGFP and its derivatives suggested that perturbation of the proteasome by the exposed cysteines of the overexpressed proteins caused cell elongation.

The less toxic moxGFP discovered during this study are expected to be better model proteins for understanding the physiological state of protein burden caused by the ultimate overexpression of harmless proteins.

235V

Candida albicans cells without Tor1 kinase N-terminal HEAT repeats are hypersensitive to cell wall stress and defective in mitochondrial function

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Target of Rapamycin Complex 1 (TORC1) makes essential decisions to direct cellular resources toward growth and proliferation in favorable conditions, or toward growth cessation and survival responses during stress. Current TORC1 inhibitors like rapamycin kill *Candida albicans*; as they target the highly conserved Tor kinase domain, they are also severely toxic to human cells.

The least conserved region of fungal and human Tor kinases is a large array of protein-protein interaction domains, the N-terminal HEAT domains. We examined the role of the 8 most N-terminal HEAT repeats of *C. albicans* Tor1 in cell wall stress. Cells that express Tor1 lacking the 8 most N-terminal HEAT repeats whose transcription is controlled by repressible *tetO* (Del381 cells) were compared with cells expressing wild type *TOR1* from *tetO* or from the native promoter. Del381 cells showed dysregulation of typical TORC1-related responses like rapamycin sensitivity and signaling in response to preferred nitrogen sources.

Del381 cells did not tolerate cell wall stress. They were hypersensitive to biosynthesis inhibitors of 2 major cell wall polysaccharides, beta-1,3-glucan and chitin. While able to grow anaerobically, these cells were severely defective in using non-fermentable carbon sources. They were hypersensitive to inhibitors of Complexes II-IV of the mitochondrial electron transport chain and had lower expression levels of genes encoding Complex I than cells expressing full length *TOR1*. Their expression profiles showed pervasive perturbations in carbon source metabolism and decreased expression of chitin- and beta-glucan biosynthetic genes.

These phenotypes were not due to nonspecific fitness losses of these cells: Del381 cells endured plasma membrane stress induced by detergent, or by membrane-disturbing antifungal agents fluconazole and amphotericin, relatively better than cells expressing full length *TOR1*. We hypothesize that the severe cell wall stress hypersensitivity of Del381 cells is due to defective mitochondrial function, leading to decreased ATP production. Nucleotide sugar building blocks of cell wall polysaccharides, like UDP-glucose (beta-glucan) and UDP-N-acetylglucosamine (chitin), require ATP in their biosynthetic pathways. Lacking their substrates, the biosynthetic enzymes are downregulated and cell wall fragility results. Disrupting N-terminal HEAT repeats of Tor1 with a small molecule might decrease fungal survival in the host, while sparing human mTORC1 function.

236V

Checks and balances of the RTG pathway under arginine deprivation and canavanine exposure in *Saccharomyces cerevisiae*

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Decades of research in *Saccharomyces cerevisiae* underlie the current dogma of mitochondrial retrograde (RTG) signaling: Rtg2-dependent translocation of the heterodimer Rtg1/Rtg3 from the cytoplasm to the nucleus induces the transcription of RTG-target genes under glutamate starvation or loss of respiration. We previously found that RTG mutants show severe growth inhibition from arginine deprivation and are highly sensitive to canavanine when grown on glucose. Here, we show that on solid media, RTG mutants are also sensitive to thialysine, a toxic lysine analog, although lysine deprivation causes a milder growth defect. Growth on an alternative carbon source restores RTG mutants' ability to grow without arginine or lysine and improves their tolerance of toxic analogs; deletion of *MIG1* affords a similar rescue on glucose and improves canavanine tolerance, except for in *rtg2Δ*. It is well known that the target of rapamycin (TOR) signaling pathway inhibits the RTG pathway. Batch growth experiments with or without TOR inhibition reveal phenotypic and regulatory differences between RTG mutants. *rtg1Δ* can sustain simultaneous canavanine exposure and TOR inhibition via rapamycin, but *rtg2Δ* and *rtg3Δ* cannot. Surprisingly, our data show that under fermentative lifestyle and arginine deprivation, both RTG signaling and TOR activity are required. This expands the universe of TOR and RTG signaling, suggesting bilateral communication rather than unidirectional RTG regulation by TOR. To the best of our knowledge, this work shows for the first time that Rtg3 activity can be separate from its role as a heterodimer with Rtg1. This work also strongly suggests a specific role for Rtg2 in canavanine tolerance.

237V

Met15 is not required for sulfur assimilation in Saccharomyces cerevisiae

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The Met15 (aka Met17 or Met25) enzyme has long been assumed to be the sole homocysteine synthase facilitating sulfur assimilation and *de novo* synthesis of sulfur-containing organic compounds (organosulfurs) from inorganic precursors in budding yeast. Here we show that an alternative homocysteine synthase encoded by the previously uncharacterized gene *YLL058W* supports growth of mutants lacking *MET15* in the absence of exogenous organosulfurs. This growth is observed specifically when cells are deposited in an automated fashion to seed colonies, but not with traditional cell propagation techniques such as thick patches of cells or liquid cultures. We show that the lack of growth in these contexts, which has historically justified the status of *MET15* as a classic auxotrophic marker, is largely due to toxic levels of hydrogen sulfide accumulation rather than an inability to perform *de novo* homocysteine biosynthesis. These data have broad implications for investigations of sulfur starvation/metabolism, including studies of aging and emerging cancer therapeutics.

238V

The transmembrane domain of fission yeast polycystin Pkd2 is essential for both its localization and function

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Polycystins are a family of conserved ion channels. Mutations of the human polycystin genes lead to one of the most common genetic disorders, Autosomal Dominant Polycystic Kidney Disease. This renal disease results in excessive kidney cell proliferation, but the underlying mechanism is unclear. The unicellular model organism fission yeast, *Schizosacchromyces pombe,* possesses a single essential polycystin homologue Pkd2 which regulates both cell growth and cytokinesis (Sinha et.al., 2022). It consists of a predicted N-terminal lipid binding domain (LBD), the central transient receptor potential like domain (TMD) and the C-terminal coiled-coil domain (CCD). In this study, we examined how the different domains of Pkd2 contribute to its localization and function by examining more than a dozen *pkd2* truncation mutants. Deletion of either LBD or TMD was lethal suggesting these domains are essential. In comparison, mutant Pkd2 lacking CCD localized exclusively on the plasma membrane with only mild cytokinesis or cell growth defects. To determine the functions of LBD and TMD, we generated chimeras by swapping the LBD and TMD between Pkd2 and its fission yeast non-essential homologue Trp663. The chimera replacing the LBD of Pkd2 with that of Trp663 rescued the temperature-sensitive mutant *pkd2-B42*. In contrast replacing the TMD with that of Trp663 failed to rescue *pkd2-B42*. The Pkd2 homologue from a dimorphic fission yeast *S. japonicus* rescued the *pkd2-B42*. However, replacement of TMD with that from either human Pkd2 homologues failed to restore the function of Pkd2. We concluded that both N-terminus and transmembrane domains of Pkd2 are required for its localization on the plasma membrane, but only TMD is indispensable for its function and the function is likely conserved within the fission yeast clade.

239V

Gene by Environment Interactions reveal new regulatory aspects of signaling network plasticity

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Phenotypes can change during exposure to different environments through the regulation of signaling pathways that operate in integrated networks. How signaling networks produce different phenotypes in different settings is not fully understood. Here, Gene by Environment Interactions (GEIs) were used to explore the regulatory network that controls filamentous/ invasive growth in the yeast Saccharomyces cerevisiae. GEI analysis revealed that the regulation of invasive growth is decentralized and varies extensively across environments. Different regulatory pathways were critical or dispensable depending on the environment, microenvironment, or time point tested, and the pathway that made the strongest contribution changed depending on the environment. Some regulators even showed conditional role reversals. Ranking pathways' roles across environments revealed an under-appreciated pathway (OPI1) as the single strongest regulator among the major pathways tested (RAS, RIM101, and MAPK). One mechanism that may explain the high degree of regulatory plasticity observed was conditional pathway interactions, such as conditional redundancy and conditional cross-pathway regulation. Another mechanism was that different pathways conditionally and differentially regulated gene expression, such as target genes that control separate cell adhesion mechanisms (FLO11 and SFG1). An exception to decentralized regulation of invasive growth was that morphogenetic changes (cell elongation and budding pattern) were primarily regulated by one pathway (MAPK). Our work suggests that GEI analysis is a simple and powerful approach to define the regulatory basis of complex phenotypes and may be applicable to many systems.

240A

Stringent genetic biocontainment in *Saccharomyces cerevisiae* by conditional stability of essential proteins

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Synthetic biology holds immense promise to tackle key problems we are facing, for instance in resource management, environment, and health care. However, comprehensive safety measures need to be developed and put into place for the use of genetically engineered microorganisms, especially for open-environment applications. Here, we describe a new genetic biocontainment system based on conditional stability of essential proteins. We used a yeast-adapted destabilizing domain degron, which can be stabilized by estradiol addition (ERdd). Leveraging the yeast GFP collection and lab automation platforms, we ERdd-tagged 775 essential genes and screened for strains with estradiol dependent growth. Three genes, *SPC110*, *DIS3* and *RRP46*, were identified to be particularly suitable. With ERdd, the respective strains showed no growth defect in the presence of estradiol and strong growth inhibition in its absence. Of these, *SPC110-ERdd* offered the most stringent containment, with an escape frequency of only 7.0×10^{-8}, and full growth restoration at 100 nM estradiol. Combining it with another ERdd tag on either *DIS3* or *RRP46* resulted in escape frequencies below the detection limit ($<2 \times 10^{-10}$). Being

based on conditional protein stability, the system we have developed is mechanistically orthogonal to previously reported intrinsic biocontainment systems. It thus can be freely combined with other systems, for instance ones based on transcriptional or translational control of essential gene expression, to achieve extremely stringent control over the survival of engineered organisms.

241A

Efficient Information Coding Over Living Organisms

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Encoding information over the genome of living organisms has various applications in synthetic biology such as biosensors, biological treatments, and very long-term storage. In addition, this topic is essential for understanding fundamental aspects of molecular evolution and genetics.

However, this topic is explicitly challenging since living organisms constantly evolve, and this can corrupt or even delete the encoded information. Unlike artificial gene synthesis, in which virtually any DNA sequence can be created, DNA synthesis in living cells imposes different mechanisms of data corruption and requires new sophisticated analysis methods.

We designed a mathematical model with efficient coding storage density, with flexible scalability, minimization of environment dependency, and decoder simplicity in terms of minimal side information needed.

Our validation experimental system uses an essential gene in *Saccharomyces cerevisiae*, *ADE13*, as a platform for DNA writing, using synonymous mutations. The experimental system includes a library of variants of the gene that will be grown with and without a chemical mutagen. The DNA changes of the *ADE13* variants will be monitored over many cell generations to estimate the impact of the changes on the fidelity of the sequences and the data gathered will be used to improve and refine our model.

242A

Application of CRI-SPA as a tool for identifying genes affecting the biosynthesis of polyketides and non-ribosomal peptides in *S. cerevisiae*

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Nature is a treasure trove of small molecules with potential applications within pharmaceutical-, food-, and cosmetics industries. The development of efficient cell factories for production of these compounds represents a challenging endeavor. *Saccharomyces cerevisiae* is commonly applied cell factory chassis and has been engineered to accommodate the biosynthesis of a wide range of small molecules including polyketides, terpenes, alkaloids, non-ribosomal peptides etc. Metabolic engineering strategies guided by genome scale models and flux balance analyses provide a path towards improving *S. cerevisiae* as a host for these heterologous compounds. However, as our understanding of gene function and cellular metabolism is incomplete, even in model organisms such as *S. cerevisiae*, attractive gene targets for cell factory improvement are likely to be overlooked in such approaches. We have recently developed a method CRI-SPA, which combines CRISPR-Cas9 induced genetic engineering with Selective Ploidy Ablation (SPA) to allow for the transfer of a metabolic pathway from a donor strain to a recipient strain library. In this study, we use CRI-SPA to examine the strains in the genome-wide gene deletion library for production of the polyketide bikaverin and the non-ribosomal peptide aspulvinone E. The two compounds were chosen for the CRI-SPA experiments, as they are both pigments. Yeast strains producing these compounds therefore display visible phenotypes to facilitate quantitation of production in a high-throughput setup. Our hope is that bikaverin and aspulvinone E may serve as proxies for production of other polyketides and non-ribosomal peptides and that based on the global understanding of, which genes influence production of bikaverin and aspulvinone E, it will be possible to devise superior metabolic engineering strategies for production of other polyketides and non-ribosomal peptides.

243A

Population genomic analysis reveals a large genomic and phenotypic diversity across South American *Lachancea cidri* strains

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Evaluating the genetic diversity of non-model species is essential for a thorough understanding of biodiversity and the complexity of the genotype-phenotype relationship in nature. In this context, the quest for new wild yeasts has increasingly gained attention because of their potential ability to provide unique attributes to fermented beverages. Patagonia offers a wide diversity of ethanol-tolerant yeasts and stands out as a bioprospecting alternative. Recently, *L. cidri* was recovered from a cider fermentation environment in Europe, Eucalyptus tree sap in Australia, and Patagonia from *Nothofagus* forests, exhibiting an interesting phenotypic diversity and biotechnological potential for wine, mead, and cider fermentations. *L. cidri* is a species phylogenetically located before the whole-genome duplication in the Saccharomycotina subphylum, with a high biotechnological potential for ethanol production and the capacity to withstand stresses related to fermentation. Here, we determined the phylogeographic history of *L. cidri* together with its biotechnological potential in fermentation. We sequenced the whole genome (shorts reads) of 55 *L. cidri* strains and a subset of them using Long-reads technology, together with an electrophoretic karyotype. The population genomics results demonstrated that South American (SoAm) strains are genetically separated from European

and Australian strains, containing more nucleotide diversity (p). Interestingly, the French reference strain belongs to the Australian lineage, with a recently dated divergence (405-51 YA), likely associated with human movements. The electrophoretic pattern showed a tremendous difference in the organization of the chromosomes between European and Chilean strains, demonstrating a high level of genomic rearrangements. These results were confirmed via *de novo* assembly, revealing a large reciprocal translocation (570 kb) between chromosome B and G in all wild isolates relative to the European reference strain, together with many other SVs. This translocation is responsible for a series of phenotypic differences between strains, some of them related to their fermentation profile. Interestingly, the wild strains exhibited great fermentation profiles under cider conditions higher than commercial strain, with low residual sugars and high ethanol levels, demonstrating a potential industrial utilization. Altogether, these results show the significant genetic and phenotypic diversity present across *L. cidri* lineages and demonstrate the importance of bioprospecting efforts in Patagonia and Australia to isolate novel wild yeast strains with extraordinary biotechnological characteristics potential for the cider and wine fermentation industry.

244A

Protein Structural Orthologs and more using Predicted Protein Structures Fred S Dietrich

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Taking advantage of the careful identification of orthologs between *Saccharomyces cerevisiae* and *Ashbya gossypii* it is possible to identify the extent to which orthologs have conserved alphafold predicted protein structure. The release of Alphafold in 2021 has opened up a whole set of questions as to how to use structure to address genetic questions. Approximately 90% of ortholog pairs between these species are mutual best matches using a widely used protein structure alignment tool, TM-align. Other methods such as foldseek give similar results. We are currently developing a more exhaustive hash based tool for searching sets of protein structure, analogous to blast but not limited to 20 structural element types as is foldseek. An example of orthologs that show up by multiple measures as not having conserved structure are the KAR1 proteins of *S. cerevisiae* and *A. gossypii.* They share ~20% protein sequence identity and contain some conserved structural aspects; thus more sensitive tools for comparing structure are needed. We are then using this information to identify potential orthologs between *S. cerevisiae* and more distantly related fungi, as a way of predicting protein function. Additionally, we are investigating the use of multiple structure alignment to improve prediction of orthologs.

Polymorphisms have long been characterized as synonymous/non-synonymous, and conservative changes/non-conservative changes. We are investigating the characterization of polymorphisms between *S. cerevisiae* strains as to what sort of structural element in the protein they occur in, and what potential changes in protein structure, hydrogen bonding, charge interactions may result from the polymorphisms.

Predicted protein structures also are potentially useful in identifying ancient paralogs within species. Multiple structure alignments appear to be quite useful in identifying the conserved structures between paralogs.

245A

Rapid, scalable, combinatorial genome engineering by Marker-less Enrichment and Recombination of Genetically Engineered loci (MERGE) Mudabir Abdullah[1], Brittany M Greco[1], Jon M. Laurent[2], Michelle Vandeloo[1], Edward Marcotte[3], Aashiq Kachroo[11] Biology, Concordia University, [2]Institute of Systems Genetics NYU Langone Health,New York, [3]Department of Molecular Biosciences, University of Texas at Austin

Large-scale genome engineering in yeast is feasible primarily due to prodigious homology-directed DNA repair (HDR), a plethora of genetic tools, and simple conversion between haploid and diploid forms. However, a major challenge to rationally building multi-gene processes in yeast arises due to the combinatorics of combining all of the individual edits into the same strain. Here, we present an approach for scalable, precise, multi-site genome editing that combines all edits into a single strain without the need for selection markers through the use of CRISPR/Cas9 and gene drives. We show that engineered loci become resistant to the corresponding CRISPR reagent, allowing the enrichment of distinct genotypes. Next, we demonstrate a highly efficient gene drive that selectively eliminates specific loci by integrating CRISPR/Cas9 mediated Double-Strand Break (DSB) generation and homology-directed recombination with yeast sexual assortment. The method enables **M**arker-less **E**nrichment and **R**ecombination of **G**enetically **E**ngineered loci (MERGE) in yeast. We show that MERGE converts single heterologous yeast loci to homozygous loci at ~100% efficiency, independent of the location on the chromosome. MERGE is equally efficient at converting and combining loci, thus identifying viable intermediate genotypes. Finally, we establish the feasibility of MERGE by engineering the carotenoid biosynthesis pathway and most of the α proteasome core in yeast. MERGE, therefore, lays the foundation for marker-less, highly efficient, and scalable combinatorial genome editing in yeast.

246A

PET plastic degradation using yeast: development of a whole-cell catalyst for MHET conversion into ethylene glycol and TPA Raphael Loll-Krippleber, Victoria Sajtovich, Mike W Ferguson, Brandon Ho, Grant W Brown Biochemistry, University of Toronto

Over the 70 years since the introduction of plastic into everyday items, plastic waste has become an increasing problem. With over 400 million tonnes of plastics produced every year, solutions for plastic recycling and plastic waste reduction are sorely needed. Recently, multiple enzymes capable of degrading PET (Polyethylene teraphthalate) plastic have been identified and engineered. In particular, the enzymes PETase and MHETase from *Ideonella sakaiensis*, have been shown to allow depolymerization of PET into the two building blocks used for its synthesis, ethylene glycol (EG) and terephthalic acid (TPA). Importantly, EG and TPA generated from PET depolymerization can be re-used for PET synthesis allowing complete and sustainable PET recycling. In this study, we used *Saccharomyces cerevisiae* as a

platform to develop a whole-cell catalyst expressing the MHETase enzyme, which converts MHET (monohydroxyethyl terephthalate) into TPA and EG. First, we assessed multiple expression architectures and identified those resulting in efficient MHETase expression. Second, we show that the yeast-expressed MHETase is active against a MHET analog substrate and displays activity comparable to recombinant MHETase purified from *E. coli*. Finally, we demonstrate that the yeast-expressed MHETase is stable across varying pH values and temperatures and over several days at room temperature. We demonstrate the feasibility of using *S. cerevisiae* as a platform for the expression of PET degrading enzymes and we predict that this cell-based system will be far more efficient than protein purification-based approaches for plastic degradation.

247A

Development of a high-throughput framework to improve the replicability of molecular biology experiments

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Improving the reproducibility and statistical power of molecular biology experiments is of key concern. We are developing a high-throughput framework that will enhance reproducibility in molecular biology experiments by using hundreds to thousands of experimental replicates while minimizing confounding factors. We will use this framework to study the nucleotide excision repair (NER) factor-I complex, which repairs bulky DNA lesions. In yeast, the NER factor-I complex is required for the survival of cells exposed to genotoxic drugs such as methyl methanesulfonate (MMS) and is encoded by the genes *RAD1, RAD10,* and *RAD14.* Homologs of these genes also exist in humans, named *ERCC4, ERCC1,* and *XPA,* respectively. While the human genes have been individually shown to function in yeast, the degree to which different combinations of yeast and human proteins can perform nucleotide excision repair is unknown. Using thousands of replicates, we will precisely quantify how interchangeable these proteins are. In our approach, we generate thousands of distinct, DNA-barcoded plasmids, each carrying one of 27 possible combinations of yeast and human NER genes. We will precisely determine the functionality of the 27 NER complex combinations by measuring the abundances of their many representative barcodes before and after MMS exposure. We see enormous potential for pooled barcoding to improve the reproducibility of everyday experiments.

248A

Efficient and Rapid Discovery of Novel Temperature Sensitive Alleles with the Onyx® Digital Genome Engineering Platform

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The advent of CRISPR based genome editing has provided a breakthrough tool for researchers throughout many biological disciplines. While innovative and effective, CRISPR based genomic editing is still faced with limitations in scalability, efficiency, diversity of edit types, and accessibility. The Onyx® Digital Genome Engineering platform developed by Inscripta Inc. addresses these limitations and more. The Onyx platform simultaneously increases the scale of genome engineering experiments and projects, while simplifying the workflow. It includes a benchtop instrument, reagents, and software solutions for edit designs and downstream analyses. Here, we present an exciting new application developed on the Onyx platform: discovery of novel temperature sensitive (TS) alleles in yeast. TS alleles are mutations in essential genes that allow for growth at permissive temperatures and restrict or reduce growth at non-permissive temperatures. While critical for the study of essential genes, TS alleles are notoriously difficult to find. Through precise, trackable genome editing, we have sought to identify novel TS alleles in two essential genes in yeast: *HEM13* and *DFR1*. Following genome engineering, selection experiments lead to the identification of many potential TS alleles in both *HEM13* and *DFR1*. Once validated, these conditional mutants can function as invaluable tools for future studies of both genes. In addition, they serve as a proof-of-concept for Onyx as a new tool for identifying TS alleles in essential genes. Collectively, this work highlights the effectiveness of Inscripta's Digital Genome Engineering platform for novel discoveries in yeast biology.

249B

Cloning, modifying, and transplanting mitochondrial genomes in *Saccharomyces cerevisiae*

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The evolution of mitochondria played a key role in the origin, success, and diversification of eukaryotic life. How this organelle arose and integrated into host cells are fundamental questions in biology. Yet, our ability to manipulate the mitochondrial genome to study its evolution and function remains limited. To address this issue, we have developed a highly efficient workflow for cloning and transplanting intact yeast mitochondrial genomes, which also enables the genetic modification and reengineering of this molecule. We are now using our approach to identify essential regions of the mitochondrial genome. Our results will guide the synthesis of a minimal, refactored mitochondrial genome, which in turn will enable synthetic genomics research on the origins of the mitochondria and the production of designer organelles.

250B

Environmental protein interaction dynamics at a proteome scale

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Protein interactions are crucial to cellular processes. Although cellular processes are known to depend on environmental context, the response of protein interactions to environmental change has not been explored at a proteome scale, in part because generating proteome-wide maps is laborious. Here we describe the massively parallel fluorescence-Barcode Fusion Genetics-Yeast Two-Hybrid (fBFG-Y2H) method and use it to query >30 million protein pairs under various contexts, including previously inaccessible environments affecting cell fitness. We produce proteome-scale yeast interactomes under baseline, DNA damage, carbon starvation, and oxidative stress, yielding the first atlas of proteome-scale dynamic protein interactome maps for any organism. Validation with an orthogonal assay shows the quality of our interactions to be on par with interactions observed at least twice in the literature. We report ~2500 high-quality interactions, ~10% of which were dynamic across conditions. To better interpret the multi-environment interactome, we obtained transcriptomic and phosphoproteomic measurements in matching conditions, capturing dynamics of cellular response to environmental perturbation. Illustrating the atlas' value, we derive an integrated model for starvation-induced filamentation of major CTP synthetase Ura7, where carbon starvation induces transcription of PKC, which in turn phosphorylates Ura7 to induce oligomerization. The atlas also revealed global trends: for example, interactions between proteins that are not members of multi-protein complexes tended to be more dynamic, and dynamic interactions tended to involve less-well-studied proteins than did static interactions.

251B

Genetic interrogation of drug-resistant *Candida albicans* essential genes during antifungal drug exposure using a novel inducible CRISPRi system

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The opportunistic pathogenic yeast, *Candida albicans* is a common inhabitant of the human microbiota capable of causing superficial to severe fungal infections in immunocompromised patients. With the emergence of antifungal resistant *Candida*. spp and other fungal pathogens, there is a pressing need for new antifungal drugs to treat these infections. To better understand how to combat these fungal pathogens, new molecular techniques provide an advantageous approach to reverse genetic studies. The proposed research outlined here describes the use of the transcriptional repression approach known as CRISPR interference (CRISPRi) to target essential genes for repression in *C. albicans* and elucidate gene function under antifungal drug exposure. A subset of 130 essential genes that were found to have homologs in three other medically relevant fungal pathogens, and lack human homologs, were chosen as candidates for this study as they represent putative broad-range antifungal drug targets. A CRISPRi pooled library targeting these genes will be synthesized and transformed into wild-type and two different clinical isolates of *C. albicans*, including strains with varying antifungal drug resistance profiles, to generate pooled fungal libraries targeted with CRISPRi. The *C. albicans* CRISPRi mutant libraries will then be subjected to competitive growth assays to identify which repression constructs are enriched or depleted after drug exposure. Specifically, we will treat the pooled fungal libraries with antifungal drugs, and determine which mutants show increased sensitivity after drug exposure. CRISPRi strains that showed increased sensitivity will be selected for follow-up phenotypic assessment to obtain a better understanding of the function of these genes during drug tolerance. Subsequently, these selected genes will be knocked down in *Cryptococcus neoformans* to compare phenotypic profiles and identify if these genes extend as candidates for novel broad-range antifungal drugs.

252B

CRISPR-Cas9 Induced Combinatorial Genome Editing in *Saccharomyces cerevisiae* for functional genomics and synthetic biology

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Baker's yeast has long served as a convenient chassis for bioengineering owing to its genetic tractability, versatile metabolism, and ease of culture in the lab. Several decades of fundamental research together with the development of high throughput toolkits and genome engineering capacities have established yeast as an ideal model eukaryote for system genetics and synthetic biology. The availability of many selectable genetic markers and simple conversion between haploid and diploid forms has provided avenues to easily combine pairs of genetically engineered loci to understand gene-gene interactions at a global scale. For more extensive genetic alterations, yeast's highly efficient Homologous Recombination (HR) pathway even enables the synthesis of entire chromosomes, although this approach requires iterative use of selection markers and tedious repetitive procedures. However, CRISPR-Cas9 allows precise editing of yeast loci without the requirement of a selection marker enabling **M**arker-less **E**nrichment and **Re**combination of **G**enetically**E**ngineered yeast loci (**MERGE**). First, we show that engineered loci become resistant to the corresponding CRISPR reagent, allowing the enrichment of distinct genotypes. The advantages of using CRISPR selection are numerous, with no requirement of markers for any number of genotypes.
Furthermore, **MERGE** converts any heterozygous yeast locus to homozygous alleles independent of the position on a chromosome. We explored the strategy across many yeast genes located on chromosome 1 demonstrating efficient conversion of heterozygous to homozygous loci, respectively. Thus, **MERGE** enables a quick screen for gene essentiality in yeast. MERGE facilitates the combination of genetically engineered loci in a fitness-driven manner enabling the engineering of humanized metabolic pathways such as the heme biosynthesis pathway and proteins complexes like the proteasome. While we provide the data for combining humanized loci, MERGE can probe higher-order genetic interactions (>3 loci) limited by the availability of selection markers. In the future, MERGE has the potential to drive new synthetic and systems biology research from engineering heterologous systems to performing multi-site and genome-wide combinatorial editing in yeast.

253B

A new reagent set to characterize the spatiotemporal dynamics of proteins encoded by temperature sensitive alleles of essential genes

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Essential genes span a wide breadth of biological functions and are hubs on the global yeast genetic interaction network. Temperature sensitive (ts) alleles of essential genes are useful reagents to probe essential gene function: at permissive temperature, mutants that carry a ts allele are viable and typically show minor growth phenotypes; however, at the non-permissive temperature, ts mutant strains are inviable (or have a severe fitness defect). Our lab previously constructed a library of ts mutants, where mutant alleles representing most essential genes replaced the wild-type version in an isogenic background. The ts mutant collection enables array-based genetics and is a powerful resource for examining essential gene biology.

While growth phenotypes of ts mutants at permissive and non-permissive temperatures have been measured, the consequences of ts mutations at the protein level have not been systematically explored. To examine mutant protein dynamics, we constructed a new reagent set of over 1000 strains, in which ts alleles of essential genes are tagged with a fluorescent marker at their endogenous loci. Using high-throughput confocal microscopy, we track each fluorescently tagged mutant protein through a 24-hour imaging time course, following the spatiotemporal dynamics exhibited by each protein *in vivo*.

Preliminary analysis of time course data for over 400 fluorescently tagged mutant proteins, each with their respective wildtype controls, revealed that over 85% of mutant proteins exhibit a phenotype during the time course. Approximately 10% of mutant proteins exhibited solely an abundance change and 15% of mutants had differential subcellular localization from wildtype. The rest of the mutants, approximately 60%, exhibited a mixture of both abundance and localization differences relative to their wildtype counterparts. Some mutant proteins localized to punctate compartments, which could represent stress-induced structures.

Using this fluorescent reagent set, we can explore the complex biology of essential genes and characterize the phenotypes that arise at the protein level, when temperature sensitivity occurs. This dataset will contribute to the growing field of phenomics, as we aim to understand the functional wiring of the cell.

254B

Creating a *Candida albicans* genome-wide CRISPR interference library for large-scale genetic analysis

Meea Fogal, Lauren Wensing, Nicholas C Gervais, Rebecca S Shapiro Molecular and Cellular Biology, University of Guelph

Candida albicans is an opportunistic pathogenic yeast that is intimately associated with humans as a commensal member of the microbiota. Infections caused by *C. albicans* can be severe and life-threatening, especially in immunocompromised individuals. Treating *C. albicans* infections is notoriously challenging due to a limited availability of safe and efficacious antifungal drugs. The emergence of drug-

resistant *C. albicans* strains further complicates treatment options. Thus, it is imperative that we study this pathogen to better understand the fundamental molecular mechanisms that underpin antifungal drug resistance. To do this, we intend to use a transcriptional repression system, known as CRISPR interference (CRISPRi), to identify previously uncharacterized genes with roles in antifungal drug resistance. We will generate a pooled CRISPRi repression library targeting each of the ~6,100 genes in the *C. albicans* genome. This pooled genome-wide CRISPRi library will be screened in the presence of antifungal drugs to identify genes that, when repressed, influence antifungal drug susceptibility. Genes of interest from this screen will be subject to follow-up phenotypic analysis and characterization to study their function more comprehensively. This research will allow us to survey all genes in the pathogen at once for their individual contribution to antifungal drug susceptibility for the first time. This library and screening platform is readily translatable and also holds potential for genome-scale functional analysis of *C. albicans* biology.

255B

A Toolkit for automated high-throughput cloning and manipulation of DNA in fission and budding yeast

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Budding and fission yeast continue to serve as outstanding models for biomedical research. While budding yeast is emerging as a model eukaryote for synthetic-biology with readily available fully-characterized toolkits, fission yeast has lagged in part due to the lack of similar resources. Furthermore, the development of Genome Foundries demands compatible platforms that enable modular, multipart cloning with precision. Here, we present toolkits combining the gateway and golden gate technologies for precise, automated, high-throughput cloning and genome engineering for both yeasts.
For budding yeast, we modified a previously available toolkit into robotics-compatible vectors. For fission yeast, this platform provides a new set of vectors for modular assemblies, including a fully characterized collection of promoters and terminators. Additionally, we engineered a non-toxic, codon-optimized, genome-editing tool for efficient modifications of the fission yeast genome. Finally, we show the utility of the toolkit for precision cloning and the expression of heterologous human proteins in yeasts.

256B

Streamlining the *Saccharomyces cerevisiae* Genome

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Our ability to predictively reprogram organisms is hindered by the complexity of natural biological systems. Here, we aim to 'streamline' the *Saccharomyces cerevisiae* genome, eliminating a large fraction of nonessential genetic elements. We are utilizing a stepwise approach, segmenting the genome into 120 partially overlapping segments of ~100kb. These genomic segments will be isolated from the *S. cerevisiae* genome using a novel *in vivo* cloning method. Once isolated, these genomic fragments will be subjected to saturating transposon mutagenesis *in vitro*, reintroduced into yeast, and assayed for viability in standard lab environments. Results from these experiments will guide the computational design and synthesis of streamlined genomic segments, which will be integrated into the genome. Completion of this work should result in an *S. cerevisiae* strain with a substantially reduced genome.

257V

A Suite of New C-SWAT Strain Construction Vectors Enables Comparative Evaluation of Essential Gene Perturbation Tools in Yeast

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Comprising nearly one-fifth of the budding yeast genome, genes essential for cell viability have roles in fundamental, highly conserved cellular processes. Although deletion of essential genes is not possible, these genes can be studied using strategies that lower their expression or perturb protein function in either a constitutive or inducible manner. To comparatively evaluate the efficacy of different essential gene knockdown and protein depletion approaches, we systematically applied seven such published strategies to a panel of essential genes encoding nuclear-localized proteins. In this effort, we created tagging vectors compatible with the C-terminal SWAp-Tag (C-SWAT) collection to improve the utility and ease of strain construction for most of these strategies. Of particular note, we adapted an improved auxin inducible degron (AID) protein degradation strategy previously available only in mammalian tissue culture for one-step strain construction in budding yeast by leveraging both the C-SWAT system and CRISPR/Cas9 editing. Expression of nearly half of panel genes was rapidly and strongly reduced in an inducible manner by the improved yeast-adapted AID system; however, the efficacy was biased toward low expressed proteins that can withstand C-terminal tagging. Conversely, the tetracycline-regulated shut-off promoter with an N-terminal destabilizing amino acid was more broadly applicable to panel genes, albeit with slower protein degradation kinetics. Taken together, this work presents a toolbox for essential gene perturbation and allows us to make recommendations on the efficacy and applicability of these tools.

258V

Conducting genetic interaction analysis in *Candida albicans* by using CRISPR-Cas9-based gene drive to target stress response genes

Viola Halder, Rebecca Shapiro University of Guelph

Candida albicans are an opportunistic fungal pathogen found in the oral mucosa, the gut, the vaginal mucosa, and humans' skin. While C. albicans can cause superficial infections, severe invasive infections can occur in immunocompromised individuals. Understanding the survival mechanisms and pathogenesis of C. albicans is critical for novel antifungal drug discovery. Determining the relationships between different genes can create a genetic interaction map, which can identify complementary gene sets, central to C. albicans survival, as potential drug targets in combination therapy. A genetic approach using the CRISPR-Cas9-based genome editing platform will focus on targeting C. albicans stress response genes for the purposes of genetic interaction analysis. The ultimate goal is to create a stress response gene deletion library to study its pathogen survival role. A small library of single and double stress response gene mutants was screened under diverse stress conditions to assess their relative fitness in terms of growth. Genetic interaction analysis was conducted to map out epistatic interactions between fungal genes involved in growth, survival, and pathogenesis based on negative or synthetic lethal genetic interactions.

259V

Direct measurement of gene-by-environment interactions through precision genome editing

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How natural genetic variation contributes to individual traits through gene-by-environment (GxE) interactions is a central question in genetics and evolution. Current methods for mapping of variants with GxE often have insufficient resolution, limiting our ability to gain insight into the molecular mechanisms of GxE. We used a novel high-throughput, precision gene editing method to measure the fitness effect of natural variants within stress conditions at single-nucleotide resolution. In our proof-of-principle experiment, thousands of natural genetic variants among diverged yeast strains were functionally assayed in a single experiment. Strikingly, we were able to observe widespread condition-specific fitness effects among hundreds of natural variants, which harbor GxE across multiple stress conditions. Moreover, we observe enrichment of missense GxE variants in some stress conditions, which we were able to resolve at base-pair resolution. Finally, we were able to identify non-coding causal variants that overlap regulatory elements, connecting gene expression to GxE. We show that this framework to detect environment-specific variant fitness effects is efficient and precise, providing molecular insights into how GxE interactions impact individual trait when challenged by environmental factors.

260A

The Yeast ORFan Gene Project: Exploring yeast genes of unknown function in the undergraduate curriculum

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Course-based undergraduate research experiences (CUREs) have numerous positive impacts on students, including increased knowledge of course content, independence, and interest in related subject matter. We describe the outcomes and assessment of a collaborative CURE that aims to explore the function of as yet uncharacterized *Saccharomyces cerevisiae* genes. Currently, of the 6611 annotated *S. cerevisiae* ORFs, a substantial number are classified as “unknown” by GO annotation: Cellular Component (1405), Biological Process (1743), and Molecular Function (2536). We hypothesized that CURE modules for study of yeast genes of unknown function can be effective tools to teach undergraduates basic bioinformatics, gene discovery tools and experimental design. Thus, we have grown a consortium of undergraduate researchers and faculty teaching in undergraduate courses that are collaborating in running laboratory modules in which students investigate genes of unknown function. Summer workshops for faculty and students introduced attendees to the bioinformatics work-flow and basic laboratory techniques and provided faculty with the knowledge and materials to incorporate the modules into courses at their home institution. Over the past six years, we have refined unknown gene selection and the laboratory techniques. Pre- and post-test assessments provide data on the effectiveness of the modules. Assessment results (n>300) confirm that students gained an understanding of the Gene Ontology (GO) system for describing gene function and knowledge in the use of bioinformatics to assign gene function. Additionally, students self-report significant gains in confidence and understanding of the scientific process. In 2022, the network concepts are being expanded to include the study of protogenes in *S. cerevisiae*.

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261A

Investigating yeast GUFs with undergraduates using virtual and laboratory approaches

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Participation in undergraduate research, a high-impact practice (HIP), offers students significant educational benefits. However, at Indiana University East (IUE), many science students do not complete undergraduate research projects due to their demanding schedules,

insufficient knowledge about the research experience, or confusion about how to obtain an independent research position. In addition, the IUE Department of Natural Science is small. Only a limited number of full-time faculty are actively involved in research. By incorporating authentic research experiences into a laboratory course, students may gain associated educational benefits without additional time input. Also, participation in course-based research experiences (CUREs) may encourage some students to pursue further research opportunities. The goal of the current work was to develop a pathway to bring the Yeast ORFan Gene Project to undergraduate students at IUE. During spring 2021, when in-person laboratory interactions were still limited due to COVID-19, we introduced a virtual research project into molecular biology lab (BIOL-L 213) and as an honors project in biochemistry (CHEM-C 485). Using bioinformatics-based research modules created by the Yeast ORFan Gene Project, undergraduate students investigated the molecular functions of genes of unknown function (GUFs) in the model organism *S. cerevisiae*. In addition to the yeast genome database (SGD), students utilized sequence alignment tools, such as BLAST-P and PFAM, and cellular localization tools and databases, such as PSORTII and the yeast protein localization database. To culminate the research, students hypothesized a potential function for their GUF based on their research and proposed a laboratory experiment that could be performed to test this function. In this initial implementation, we found significant post-course gains in confidence in the process of science and in experience with research. In particular, the largest gains were observed in the students' experience with research projects that are student-driven and open-ended in nature. Since the completion of the course, one student has continued research on their GUF, YJL206C, in the laboratory, and results of this independent research will be presented, too. Work was supported in part by NSF grant #1624174.

262A

Student exploration of trinucleotide repeat expansion control by genes of unknown function in Saccharomyces cerevisiae

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The Yeast ORFan Gene Project (NSF RCN UBE #1624174) is a collaborative Course-based Undergraduate Research Experience (CURE) that aims to explore the function of as yet uncharacterized *Saccharomyces cerevisiae* genes. In a collaboration between Ohlone College and California State University San Marcos, we are working to identify genes of unknown function that might contribute to trinucleotide repeat expansions, such as those associated with Huntington's disease. The *RAD27* gene has been associated with genome maintenance and repeat expansions. Using the ORFan Gene Project bioinformatic modules, students in our laboratory courses identify genes of unknown function with similar Gene Ontology (GO) terms to the yeast gene *RAD27*, or genes that interact or are co-expressed with *RAD27*. We then use CRISPR-Cas to delete the selected genes, providing cells with a homology directed repair (HDR) template that is engineered with a premature stop codon to prevent gene expression. This instructional approach enables students to see the versatility that can be achieved with gene editing. Using the Saccharomyces genome database resources, students design gRNA and HDR repair templates, and then use PCR to clone the gRNA. Yeast strains are then transformed with the student generated plasmids and HDR templates. The putative deletion strains are confirmed using PCR and restriction enzyme digest, looking for the loss of an endonuclease recognition sequence in the HDR template. Time permitting, Sanger sequencing could also be done to confirm the deletions. Spot assays are used for a phenotypic analysis of the deletion strains to a control strain, with and without exposure to UV light. Posters are prepared and presented by the student teams as their final exam project. We also have a yeast strain containing a triplet-repeat cassette in one of its chromosomes that can be tested in a PCR assay, perhaps in a future semester not plagued by ransom-ware attacks, COVID outbreaks and supply chain troubles.

263A

Teaching genetics with yeast domestication case studies

Bryce Taylor Loras College

The yeast *Saccharomyces cerevisiae* has been our companion in food and beverage production for at least 9,000 years. It has played an important role in the global food supply, diverse religious traditions, and the development of the field of genetics. It's also arguably the most well-characterized organism at the genetic level. This has made it possible for geneticists to identify specific genetic factors that played a role in yeast's proclivity for fermentation, including mutations that our ancestors selected for during the domestication process. The combination of cultural history and molecular genetics makes case studies in yeast domestication a powerful tool for genetics curricula. In this presentation I will share my strategies for incorporating these case studies into standard genetics curricula and resources I have developed for teaching them in the classroom. I will additionally share a framework for using these case studies to frame open-ended yeast genomics research modules in a lab course setting (a CURE). This framework sets students up to carry out research into yeast domestication by (1) analyzing whole-genome sequencing data to look for domestication alleles, and then (2) make predictions about the phenotypes of sequenced yeast based on their genotype.

264A

Build-a-Genome: Cutting-edge concepts using simple model organisms

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The Build-a-Genome course, initiated at Johns Hopkins University, provides a comprehensive introduction to synthetic biology, including gene and genome synthesis, the creation of genetic devices and designer organisms, and ethical considerations in genetic engineering. In this course-based undergraduate research (CURE) opportunity, students learn about genome structure either by building a portion of the *Saccharomyces cerevisiae* genome, by creating a neochromosome, or by performing induced genome rearrangement of yeast cells followed by Illumina sequencing. In addition to introducing novel concepts of synthetic biology into the undergraduate curriculum in an accessible way, the course offers students a truly authentic laboratory experience by prioritizing a set of molecular biology techniques (PCR, gel electrophoresis, microbial cell culture, transformation) with which students become technically fluent; they also design and troubleshoot their own experiments and present data at frequent interactive lab meetings. Now in the 4th year as a national network, the Build-a-Genome Network has been established at large research universities, mid-sized comprehensive universities, small liberal arts colleges, and

community colleges by making adaptations to this research-intensive course, addressing the challenges that are encountered in different academic environments, and finding ways to overcome the constraints on time and resources often encountered at undergraduate institutions. The network has developed resources to introduce synthetic biology concepts through lectures, to develop skills with web-based bioinformatics tools, to increase student technical skills and self-efficacy in the laboratory, and to engage students with case studies on issues of genetic manipulation as it relates to human health and environmental conservation. Student feedback and experimental results indicate that the most attractive pedagogical aspects of Build-a-Genome course are maintained across diverse institutions, and that this authentic laboratory experience impacts student attitudes and enthusiasm for research.

265B

Educational Resources Hosted at the *Saccharomyces* Genome Database.

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The *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org) is the leading community resource for the budding yeast *S. cerevisiae*. SGD provides high-quality, manually curated information on the yeast genome and offers a wide variety of tools and features that make it an indispensable resource for researchers. SGD engages in a variety of online training and educational outreach efforts to inform our user community about new developments, to improve user familiarity with SGD features and tools, and to increase public awareness of the importance of yeast not only for biological and biomedical research but also for instructional purposes.

The SGD community wiki (https://wiki.yeastgenome.org) provides users with a venue for accessing and sharing information in areas that include educational resources. This includes information about associations and societies, general and yeast specific classroom materials (teaching modules and project-based courses), and some fun sites of general interest to the aspiring biologist.

To inform the community about new features and tools, SGD creates and posts short videos to YouTube (https://www.youtube.com/SaccharomycesGenomeDatabase) as a means to both educate our users and address questions posed by users. This includes videos on how to use tools like: YeastMine, Variant Viewer, GO Term Finder, GO Slim Mapper and JBrowse, as well as videos to support users interested in navigating phenotypes, interactions, expression data, literature, homologs, human disease connections and functional complementation.

SGD is also working with micropublications.org to promote the publication of brief, novel, technically sound research results and data that don't fit into full-length articles. This includes single high-quality research results as well as negative results that will accelerate scientific discovery and advance the scientific endeavor. This mechanism for publication is particularly attractive for students interested in rapidly publishing findings of general interest to the greater scientific community. Micropublications are indexed at PubMed, PubMed Central (PMC) and EuropePMC for greater visibility.

We will continue to develop these services to provide access to educational resources and outreach for students, teachers and scientists to facilitate greater use and understanding of the resources made available by SGD. This work is supported by a grant from the NHGRI (U41 HG001315).

266B

***In vivo* Characterization of the Critical Interaction between the RNA Exosome and the Essential RNA Helicase Mtr4**

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Using a budding yeast model to explore the consequences of novel disease mutations that occur in evolutionarily conserved genes is a valuable approach to engage junior scientists in research. The work described here constitutes the honors thesis of an Emory undergraduate student exploring a mutation found in multiple myeloma. RNA processing mutations are the second most common class of mutations linked to multiple myeloma, and mutations in the 3'-5' riboexo/endonuclease gene, *DIS3*, have been repeatedly identified in patients with this disease. DIS3 associates with the RNA exosome, a conserved, exo/endoribonuclease complex that processes/degrades numerous coding and non-coding RNAs. The 10-subunit core RNA exosome is composed of three S1/KH cap subunits (yeast Rrp4/40/Csl4; human EXOSC2/3/1), a lower ring of six PH-like subunits (yeast Rrp41/42/43/45/46/Mtr3; human EXOSC4/7/8/9/5/6), and the catalytic subunit, Rrp44/DIS3. Recently, a rare multiple myeloma patient missense mutation was identified in the cap subunit gene *EXOSC2*. This missense mutation results in a single amino acid substitution, M40T, in a highly conserved domain of EXOSC2. Structural studies suggest this M40 residue makes direct contact with the essential RNA helicase, Mtr4, and may help stabilize the critical interaction between the RNA exosome complex and this RNA helicase. To assess this interaction *in vivo*, we utilized the *Saccharomyces cerevisiae* system and modeled the mutation *EXOSC2 M40T* into the orthologous yeast gene *RRP4*, generating the variant *rrp4 M68T.* The *rrp4 M68T* model showed no growth defect; however, when challenged with drugs or combined with RNA exosome cofactor mutants we detect a significant decrease in growth rate. Additionally, we identified a strong and specific negative genetic interaction between the *rrp4 M68T* cells and *mtr4* mutant variants. Furthermore, qPCR analysis shows accumulation of specific target RNAs in *rrp4 M68T* cells, suggesting a destabilization of the interaction between the helicase and the RNA exosome complex. These experiments provide the first characterization of this multiple myeloma mutation as well as an *in vivo* approach towards assessing this critical interaction between the RNA exosome and Mtr4. These studies were performed in a collaboration between the Emory College Biology Department and the Winship Cancer Institute at Emory University, representing the intersection between undergraduate mentorship and exploratory cancer biology research.

267B

yEvo Lab: A University-High School Collaboration to Evolve Caffeine Tolerance in Yeast

Naomi G Moresi, Renee C Geck, Maitreya J Dunham Genome Sciences, University of Washington

yEvo was developed as a curriculum to perform laboratory evolution experiments with high school students. The first iteration of this curriculum evolved azole-resistant yeast. yEvo is now expanding our curriculum to other selective pressures including caffeine. Caffeine is a relevant selection factor as yeast are used in the food industry to ferment foods containing caffeine, and studying caffeine helps increase understanding of TOR (target of rapamycin) signaling, which is inhibited by caffeine.

TOR signaling is a nutrient-sensitive pathway that plays a role in cell growth and aging. Caffeine inhibits TOR signaling and growth, compromising overall cellular fitness. Yeast allows us to study TOR signaling using experimental evolution, where under a defined selective pressure - here, caffeine - we observe what rare beneficial mutations arise and increase in frequency.

While many inputs to the TOR signaling pathway are known, others are yet to be identified. Furthermore, how other pathways compensate for TOR signaling inhibition is not completely understood. Our goal is to identify factors involved in TOR signaling by growing yeast in inhibitory concentrations of caffeine to select for better-growing mutants with increased caffeine tolerance.

We collaborated with classrooms at 2 high schools to evolve 50 caffeine-tolerant yeast strains. Students grew yeast for 5-10 weeks in increasing doses of caffeine, and expressed satisfaction in being able to see growth improve over time and master sterile techniques. Additionally, participating classrooms used yeast strains expressing colored pigments enabling students to mix and compete strains to determine which strain was most fit in caffeine.

We sequenced the genomes of resistant strains from student evolution experiments and university pilot experiments, and are studying the resultant mutations to determine how they connect to TOR signaling and caffeine tolerance. We confirmed that evolved yeast had increased caffeine tolerance, as they had higher growth rates in media containing concentrations of caffeine that inhibited growth of the ancestral strains. We identified mutations in drug-response pathways including in the Pdr1 transcription factor. We also observed mutations in processes regulated by TOR, such as nutrient sensing. As we continue to sequence more evolved populations we aim to identify novel mutations and factors involved in caffeine tolerance and TOR signaling. Ultimately, these findings increase our understanding of how caffeine impacts TOR signaling and how other cellular processes are regulated by TOR. More broadly, this research can aid in the continued development of how cell signaling pathways are related to nutrient response, aging, and growth, and enables high school students to master techniques, better understand evolution, and participate in research and discovery.

268B

Investigating Genes of Unknown Function: Student Experience

Claire E Magill[1], Hailey H Hendricks[1], Jill Keeney[21]Juniata College, [2] Biology, Juniata College

Despite decades of research into the Saccharomyces cerevisiae genome, there are close to a thousand genes of unknown function. This is not due to the lack of effort from researchers, but from the possibilities of genetic redundancy and the lack of strong growth phenotype. Determining genes of unknown functions of yeast will allow us to better understand the functional relationships between genes and the yeast genome and apply it to larger organisms. The Yeast ORFan Gene Project is an undergraduate and faculty research project to determine molecular functions of genes with unknown functions in Saccharomyces cerevisiae. At Juniata College, undergraduate students have the opportunity to get involved with research that provides valuable learning outcomes that apply to future careers within research and healthcare. One of the verified ORFs chosen was FVY8 (YGR196C). The known information about FVY8 from Saccharomyces Genome Database describes it as a protein of unknown function required for survival upon exposure to K1 killer toxin that is localized to the cytoplasm. To better understand the location and function of FVY8, bioinformatic modules were completed and revealed that FVY8 may have mitochondrial targeting signals, as well as a coil structure suggesting structural protein or DNA-binding protein structures. It was hypothesized from the findings that FVY8 may be involved in the specific protein pathway that degrades killer toxins. The chosen gene was deleted to create an isogenic deletion strain of the wild type strain in an attempt to determine potential functions and phenotypes of FVY8. Growth on calcofluor white suggests preliminary evidence for cell wall involvement of FVY8. Another one of the verified ORFs chosen was FUN19 (YAL034C). Currently, its molecular function, biological process, and cellular components are unknown. To better understand the location and function of FUN19,bioinformatic modules were completed and suggest that the localization of the FUN19 protein may be nuclear. The FUN19 ORF was deleted, and growth in the inhibitor 2-deoxy-D-glucose was compared with the isogenic wild type strain. The localization of the protein will be investigated by fluorescently-tagging FUN19.

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269B

A Yeast CRISPR Course-Based Undergraduate Research Experience (CURE)

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Traditional undergraduate research experiences within the laboratory of a faculty member have been associated with positive student outcomes. However, these opportunities are potentially limited by the capacity of faculty members to mentor students, and by the capability of individual students to be able to volunteer. Course-based undergraduate research experiences (CUREs) have a goal of using traditional teaching laboratories to deliver many of the benefits of an inquiry-based research experience at a larger scale and in a more equitable manner. Yeast is an ideal model organism to deploy in CUREs due to the low-cost, rapid growth, and relative ease of experimental use. We have developed a yeast CRISPR based CURE for an undergraduate biochemistry teaching laboratory. The students are provided with a database of human cancer associated mutations, where they develop their own hypothesis regarding the effects of specific conserved missense mutations on the activity of an alkaline phosphatase enzyme. The students then engineer their desired mutation into the yeast genome using CRISPR/Cas9. The resulting novel yeast strain is then used to perform a simple colorimetric biochemical enzyme assay to test the student hypothesis. The current yeast CRISPR CURE is cost effective, robustly developed with freely available detailed protocols, and is focused on a biochemistry teaching laboratory. However, the underlying CURE approach of using student generated hypotheses, CRISPR/Cas9 genetic engineering in yeast, and assaying an economical and easy to measure phenotype is widely applicable and could be deployed in a variety of biological teaching labs.

270V

Gene expression hands-on classes for undergraduate students with SGD and RSA-Tools databases

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The *Saccharomyces cerevisiae* SGD (1) and RSA-Tools (2) databases have been used to study of Gene Expression in Yeast with undergraduate Biology students. The topic is Biochemistry and Molecular Biology and the teaching involves Master classes, seminars and practical classes. It si designed for third-year course students having a prior knowledge of Genetics and Biochemistry

In the practical classes the students have to work with a Yeast gene of unknown function and extract information from the databases. This information should allow them to figure out the possible functions based on the extracted information as the type of protein interactions, the domains detected or the regulatory elements present in the gene promoter. With this last information they have to propose a hypothesis with one possible transcriptional regulator and then an experiment that could provide evidence that the hypothesis is correct.

Temporary organization: it is organized in two face-to-face sessions followed by a temporary period for students to work on the subject. They to attend a tutorial before submitting a portfolio of the complete work.

Class dynamics: The students are provided with a Word template in which they have to search and paste and/or perform what is indicated to them. To help them in the process, the teacher begins the work with SGD and the *ADH1* gene (as an example). Explanations and searches alternate with the model gene followed by the students' work with their gene of unknown function. The experience indicates that alternation teacher-students is more efficient than to have a first class with teacher explanations and a second one with student’s searches.

SGD gives, among others, information about transcriptional regulators, however, the RSA-Tools is more immediate to obtain the promoter or the downstream regions of the given yeast gene, as well as search for known binding sites at the promoter. The obtained information combined with the SGD functional explanation of the particular regulators as Gal4 (if it appears), will be used to propose the transcriptional hypothesis.

This course has also been taught online during confinement by SARS-Cov2. As we used the Teams Platform (the institutional one) the teacher can help the students allowing them to share their screen and therefore to solve their problems and also do the tutorial personal class needed before the work delivery.

The final assessment takes into account, i) the full completion of the template, II) The interpretation of protein interactions and iii) The quality of the experimental model: originality of the proposal, methodological correctness and good justification of the expected result.

1-SGD: Saccharomyces Genome Database https://www.yeastgenome.org/

2-RSA-tools: (Regulatory Sequence Analysis Tools). http://embnet.ccg.unam.mx/rsat/?msclkid=61b340f8c6e611ec829428fc9c712516