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ABSTRACT BOOK

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Living with a killer: how coevolved *Saccharomyces cerevisiae* become killer toxin resistant

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Some yeasts are killers. They secrete toxins that kill neighboring cells but protect themselves with an intracellular antidote. Killers have an advantage in heterogeneous populations, although toxin production comes at a metabolic cost. Many *Saccharomyces cerevisiae* strains require two viral genomes to be killers. Along with sensitive cells in the environment, this is a complex genetic conflict: yeast and viral genomes alike are capable of adaptation and fitness tradeoffs abound at many levels. Our research dissects the molecular basis of adaptation in the face of these competing selection pressures to understand evolutionary outcomes.

We identified beneficial mutations that arose in coevolved killer and sensitive yeast, including mutations responsible for toxin-resistance. Through bulk segregant analysis, we identified a putative gain-of-function missense mutation in the HOG osmoregulatory pathway component *SSK1*. This dominant-acting polymorphism is sufficient to protect yeasts from the coevolved toxin, and other killer toxins, but comes with collateral sensitivity tradeoffs to other anti-fungal treatments. *SSK1* is a member of a two-component system, which are not found in metazoans, and has been implicated in fungal adaptation under other conditions. This data supports two-component systems and SSK1 in particular as appealing antifungal drug targets.

SSK1 is required for multiple drug resistance in *Candida auris*, and our ongoing work will address how this mutant allele might impact phenotypes of fungi associated with human pathogenicity and drug resistance.

Our preliminary data suggest the evolved *SSK1* mutation may affect protein-protein interactions, leading to crosstalk with the Cell Wall Integrity (CWI) pathway. We are currently pursuing the transcriptional and signaling impacts of the toxin-resistant allele, as well as profiling other evolved toxin resistant mutations to understand tradeoffs during adaptation. In future, we aim to understand how killer yeasts counter-adapt in the face of resistant competitors, and how viral genomes evolve as competing host population composition changes. Understanding how fungi adapt, defend themselves, and kill one another has important implications for health during climate change, as fungal pathogens emerge and drug resistance spreads.

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Spt5's central KOW domains and the Pol II Stalk Collaborate to Regulate Chromatin and 3'-End Processing

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Spt5 is a universally conserved multi-domain transcription elongation factor that functions as a component of all RNA polymerase II (Pol II) elongation complexes. Structural studies indicate that several of Spt5's central KOW domains lie adjacent to the Pol II stalk, composed of the reversibly dissociable subunits Rpb4 and Rpb7. However, the in vivo functions of these KOW domains are unknown. Here we show that Spt5 and Rpb4/7 jointly modulate 3'-end formation and co-transcriptional chromatin integrity in Saccharomyces cerevisiae. We identify mutations in the SPT5 KOW2-3 domains and RPB7 that cause cryptic initiation of transcription and alter 3'-end formation. Allele-specific genetic interactions between SPT5 and RPB4/7 reveal functional cooperation, with double mutants exhibiting synthetic defects. Molecular read-through assays demonstrate allele-specific termination defects at both GAL10 and SNR13, consistent with impacts on polyadenylation-dependent and noncoding transcript termination. Proteomic analysis of isolated Spt5 KOW2-3 enriches factors from both of these termination pathways as well as chromatin regulators, overlapping known Rpb7 interactors. Finally, single-molecule optical tweezers assays using a stalkless Pol II reveal altered pause positioning and barrier height during transcription through the nucleosome, consistent with a role for Rpb4/7 in chromatin transcription. These findings support a model in which the Spt5 KOW2-3/Pol II stalk region functions as a regulatory hub coordinating pre-mRNA processing and chromatin dynamics during elongation, revealing new roles for both Spt5's central KOW domains and the Pol II stalk.

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

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Genetic suppression occurs when the deleterious phenotype of a mutation can be rescued by another genomic alteration. Systematic analyses of suppression interactions in yeast and other model organisms have provided insights into the properties of genetic suppression and uncovered relationships between genes and pathways. However, these studies primarily relied on deletion alleles to identify suppression interactions, while missense mutations can often be suppressed through additional mechanisms that are not detected with deletion alleles. Here, we used a collection of 1,179 temperature sensitive (TS) mutants of 728 essential yeast genes to isolate spontaneous suppressor mutations that rescued the TS phenotype. We could isolate suppressors for ~90% of the tested alleles, suggesting that nearly all deleterious point mutations can be suppressed. Whole-genome sequencing of the ~2,500 isolated suppressor strains identified the mutations underlying the TS phenotype as well as candidate suppressor mutations. The TS mutations often affected protein stability and occurred at highly conserved sequences or on interaction interfaces. Most suppressors highlighted new connections between genes. In addition to interactions among functionally related genes (~70%), ~10% of suppressors involved general suppression mechanisms that affected the expression or stability of the TS mutant mRNA or protein, and ~20% of suppressor mutations occurred in the TS allele itself, restoring protein function. Finally, we explored the functional consequences of the TS mutations to pinpoint suppression interactions that are specific to the disruption of a particular protein subdomain. For example, we found that increased dosage of *DSN1*, encoding an interaction partner, suppresses mutants of the kinetochore protein Spc24 in an allele-specific manner, suggesting that this suppression depends on the proximity of the TS mutation to the interaction

interface. This work is generating the most extensive global suppression network for any organism, identifying novel functional connections between genes, and improving our understanding of how mutations can interact to produce unexpected phenotypes.

4

How the N terminal, unstructured region of a synaptonemal complex protein couples two hallmark features of meiosis

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The accurate segregation of chromosomes during meiosis relies on the prior establishment of at least one connection between homologs (mom's and dad's version of each chromosome) and such connections are provided by reciprocal exchange of DNA duplexes (crossover recombination) in conjunction with sister chromatid cohesion. Crossovers rely on several "ZMM" factors (Zip2, Zip3, Zip4, Spo16, Mer3 and MutSγ) that collaboratively engage the recombination intermediate, and such ZMM-DNA ensembles exist within the physical context of a unique supramolecular protein assembly called the synaptonemal complex (SC). Although distinctly different from one another in form and function, the processes of crossover recombination and SC assembly are physically and functionally linked, and our research is aimed at deepening our molecular understanding of how ZMM and SC proteins "play" with one another and with DNA to ensure the formation of homolog connections. We previously discovered that the N terminal tip of a major SC structural component (Zip1) contains adjacent domains that independently function to promote crossovers or SC assembly, we speculated that adjacency between these domains is mechanistically important for linking SC assembly to an intermediate step in recombination intermediate processing. We have now determined that the crossover-promoting region of Zip1's N terminal tip directly interacts with a conserved pro-crossover RING protein, Zip3. Interestingly, our structure-function analysis suggests this Zip1-Zip3 interaction both promotes crossovers and ensures SC assembly is properly coupled to recombination. We have moreover uncovered evidence for the idea that phosphorylation of Zip1's N-tip coordinates its dual functions: Replacing serines 8 and 9 with alanine abolishes Zip1's capacity to assemble SC but preserves its crossover function, whereas the Zip1[S8D, S9E] protein builds SC but is crossover-deficient. Experiments that conditionally induce Zip1-YFP show that the [S8A,S9A] alteration renders Zip1 incapable even of incorporating into previously built SC. Thus, phosphorylation of serines 8 and 9 may be a "molecular switch" that toggles Zip1 between its two critical meiotic roles, possibly underpinning how an intermediate step in the processing of a DNA joint molecule might trigger SC elaboration along the length of a chromosome pair.

5

SUMO Augments the Meiotic DNA Damage Response

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Protein modification by the ubiquitin-related modifier, SUMO, helps orchestrate the complex events of meiotic prophase I, including chromosome pairing, synapsis, and recombination. Our proteomics analysis previously identified cohorts of SUMO-modified proteins in budding yeast involved in each step of meiotic prophase I. A prominent SUMO target is the conserved HORMA-domain protein, Hop1HORMAD1, a dynamic component of chromosome axes that mediates interactions between homologous chromosomes. Hop1 promotes: (i) the formation of DNA double-strand breaks to initiate recombination; (ii) recombination between homologous chromosomes rather than sister chromatids (inter-homolog template bias); and (iii) DNA-damage checkpoint signaling. We show that a SUMOylation-deficient mutation, hop1-15KR, does not impact DSB formation or inter-homolog bias, but causes a specific defect in DNA-damage checkpoint signaling. Specifically, hop1-15KR cells fail to arrest when DNA breaks cannot be repaired because they are defective for activation of the effector kinase, Mek1CHK2.

To independently corroborate a role for SUMOylation of Hop1 in the meiotic DNA-damage checkpoint response, we adapted an orthogonal approach to target removal of SUMO from Hop1 in real time. The SUMO isopeptidase, Ulp1, was fused to the GFP-binding protein (GBP, a single-chain nanobody that binds GFP) and placed under the control of the cyanamide-inducible PDDI2 promoter. Confirming that SUMOylation is important for the checkpoint function of Hop1, targeting GBP-Ulp1 to Hop1-GFP allows cells to bypass arrest when DNA breaks cannot be repaired.

Mechanistically, we show that SUMOylation stabilizes Hop1 by impeding proteasomal degradation. Our analysis reveals a new facet of Hop1 regulation that specifically augments its meiotic DNA-damage checkpoint function.

6

Optimized yeast-based assay system to study meiotic/germ cell recurrent copy number variation

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De novo recurrent copy number variations (CNVs) are an important source of genomic disorders in humans, including a range of neurodevelopmental conditions. Recurrent CNVs are thought to occur through meiotic non-allelic homologous recombination (NAHR) between large directly oriented low copy repeats (LCRs) that are present in the human genome. NAHR mediated by these repeats results in duplications or deletions of the interstitial segment between the LCRs, and disorder phenotypes may result when dosage-dependent genes are present within those regions. We have developed and optimized an assay system to study NAHR in *Saccharomyces cerevisiae* to investigate the conserved pathways that govern the formation of *de novo* recurrent CNVs. Our system takes advantage of the fact that diploid yeast cells undergoing meiosis form tetrad structures, which physically hold together the four sibling haploid cells derived from a single meiotic division. We manipulated yeast chromosomes to contain engineered pairs of LCRs flanking CNV reporter cassettes of two types: phenotypic growth markers and genes encoding spore-autonomous fluorescent proteins. We have used this system to directly detect, quantify, and characterize meiotic haploid cells carrying recurrent CNVs through tetrad dissection and haploid cell growth. In addition, we have demonstrated the ability to indirectly quantify the presence of CNV in intact tetrads through fluorescence microscopy. We have now successfully validated and characterized this experimental assay system at two different chromosomes in wild type and mutant yeast. We have interrogated CNV formation for an initial set of meiotic function genes, finding at least one knock out mutant that displays significantly increased CNV frequency, and an altered qualitative spectrum of CNVs. This assay system is now ripe for follow-up experiments in which we will explore the endogenous and environmental factors that modulate the frequency of *de novo* recurrent CNV formation in this simple eukaryotic model.

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Genome mutagenesis by Rad5 variants in *S. cerevisiae*

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Mutagenesis promotes both genetic diversity and disease progression. A major source of mutagenesis is translesion synthesis (TLS), where lesions that block DNA replication are bypassed in an error-prone manner. In *S. cerevisiae*, Rad5 is involved in both error-prone TLS and error-free template switching, and a natural variant of *RAD5* causes increased mutagenesis as a result of TLS. How Rad5 regulates repair choice and contributes to genetic diversity remains to be understood.

To understand the role of Rad5 in modulating mutagenesis, we analyzed *RAD5* sequences from 2000+ *S. cerevisiae* isolates, revealing extensive diversity across the protein sequence, with the functional domains being conserved. Using hydroxyurea (HU) sensitivity as a proxy for Rad5 function, we performed a HU competition screen with a library of *RAD5* natural variants and identified two alleles that display HU sensitivity and increased mutation rate. We found that while the elevated mutation rates in both alleles are driven by rare variants in the *RAD5* ATPase domain, the causal variants modulate mutagenesis through distinct mechanisms. Finally, we identify a brewery strain carrying a mutagenic *RAD5* allele that has a 10-fold increase in mutation rate. Taken together, we present a framework for leveraging natural variants to study mechanisms of mutagenesis, and highlight the importance of Rad5 in contributing to genetic diversity through mediating DNA repair pathway choice.

8

Incompatibilities between*MLH1*and*PMS1* affect DNA mismatch repair function and are suppressed by a network of co-evolving polymorphisms

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Elevated mutation rates can enable microorganisms to more rapidly adapt to environmental stress but can also lead to long-term fitness defects. Previously we identified a mismatch repair (MMR) defect, referred to as an incompatibility, in laboratory strains of baker's yeast expressing *MLH1* and *PMS1* genes derived from S288c and SK1 strains, respectively. This work led to a model in which transient elevations in mutations rates can be beneficial if buffered by mating. To better understand the molecular mechanisms that underlie this incompatibility, we analyzed 3,034 sequenced and phylogenetically organized baker's yeast genomes. Two low frequency polymorphisms (*E393, N681*) were identified in *MLH1* that align with alanine scan mutations that dramatically enhance the $MLH1_{S288c}$-$PMS1_{SK1}$ incompatibility. These two polymorphisms have co-evolved in a clade of Mexican Agave yeast that display extensive introgression with *S. paradoxus*. Interestingly, *mlh1-E393* maps to a conserved motif in the intrinsically disordered region of Mlh1 that is required for Mlh1-Pms1 endonuclease activity. The *mlh1-E393* polymorphism also displays incompatibility with $PMS1_{SK1}$ and significantly reduces $Mlh1_{S288c}$-$Pms1_{SK1}$ endonuclease and recycling activities. The mutator phenotype conferred by the *E393* polymorphism is suppressed to different extents by *MLH1* (*P*271, *N681*) *and PMS1 (R818)* polymorphisms in natural populations. We present a model in which independent combinations of *MLH* polymorphisms that disrupt MMR can arise in nature but are suppressed by both mating and the presence of compensatory *MLH* polymorphisms.

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Impacts of Replication-Coupled Chromatin Assembly Pathways on DNA Replication.

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The DNA replication machinery must not only accurately initiate DNA replication and then duplicate genetic information each S phase but also navigate through features that impede fork progression to maintain genome integrity. A network of alternate replication-coupled chromatin assembly pathways assists the DNA replication machinery with these tasks by disassembling different forms of chromatin in front of the replication fork and then rebuilding appropriate nucleosomes behind the fork to ensure memory of which transcriptionally active or silenced chromatin states must be preserved throughout the duplicated chromosomes for the next cell generation. While great strides have been made in recent years in identifying factors involved in DNA replication or chromatin assembly as well as biochemical, enzymatic or molecular functions of these factors, how alternate pathways within this network divide this labor and coordinate across the genome are less well understood. To decipher where alternate replication-coupled chromatin assembly pathways play key roles during DNA replication at near base pair resolution, replication profiles were assessed in cells with defects in the CAF-1 or *RTT106*-dependent chromatin assembly pathways relative to wild-type in both the presence and absence of DNA replication stress. Replication phenotypes associated with defects in individual or multiple pathways will be highlighted to illustrate how alternate pathways differentially impact the efficiency of DNA replication initiation, fork progression, replication termination and replication timing as well as the nature of sites where replication forks have a propensity to stall or collapse when chromatin assembly is compromised.

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Primed to Burst: Corepressors Coordinate Transcriptional Activation and Efficient Switching Between Cell States

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Recapitulation of the nuclear phytohormone auxin response pathway in yeast led to the hypothesis that corepressors prime loci for rapid bursts of transcription. Specifically, the TPL corepressor, which inhibits transcription in the absence of auxin, interfaces with Mediator and core components of the transcription pre-initiation complex (PIC). Once auxin is perceived, RNA Pol-II is likely to essentially displace TPL, allowing for rapid transcription initiation (and likely re-firing). The current evidence supports a role for TPL-type corepressors in multiple levels of transcriptional control, including modulation of regulatory transcription factor activity, interactions between cis-elements and the core promoter, and in organizing multiple loci within the nucleoplasm. We have leveraged a synthetic biology approach and two unbiased whole-genome approaches to map the physical and genetic interactions of TPL at a repressed locus. We identified SPT4, SPT5, and SPT6 as necessary for repression with SPT4 acting as a bridge connecting TPL to SPT5 and SPT6. We discovered the association of multiple additional constituents of the transcriptional preinitiation complex at TPL-repressed promoters, specifically those involved early in transcription initiation. These findings were validated across eukaryotes (yeast, plants and metazoans). Our findings support a model where TPL nucleates preassembly of the transcription activation machinery to facilitate the rapid onset of transcription once repression is relieved.

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Combinatorial control of RNA polymerase II transcript synthesis by yeast nuclear RNA-binding proteins

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At least five essential nuclear RNA-binding proteins in yeast aid the synthesis and processing of RNA polymerase II (RNAP II) transcripts: Npl3/Nab1, Nab2, Nab3, Hrp1/Nab4, and Nrd1. Npl3, Nab2, and Hrp1 function mostly in mRNA synthesis, although Hrp1 can also promote short noncoding RNA termination. Conversely, Nab3 and Nrd1 function mostly in short noncoding RNA termination by RNAP II but can also regulate mRNA synthesis by attenuation (premature termination).

Our recent studies focus mainly on Hrp1 and Nrd1 function in the Nrd1-Nab3-Sen1 (NNS) termination pathway. Hrp1 appears to function as an antitermination factor for all RNAP II transcripts. Binding to specific sequences in the nascent transcript may release Hrp1 from the RNAP II elongation complex (EC), slowing transcription and favoring termination. Hrp1 functions mainly at mRNA cleavage and polyadenylation sites but also at some NNS terminators. It autoregulates its mRNA via attenuation and is required for efficient termination of snR82 small nucleolar RNA. Substitutions in the RNA-binding surface of Hrp1 usually cause terminator readthrough, whereas substitutions in other regions of Hrp1 can cause premature termination, possibly by weakening its binding to the RNAP II EC.

It is not yet clear how binding of Nrd1 to the nascent transcript induces termination. Human and fission yeast orthologs of Nrd1 have been implicated in antitermination but neither organism is known to have an NNS-like termination pathway. Budding yeast Nrd1 may compete with Hrp1 for binding to the RNAP II EC and stabilize a termination-competent conformation. Nrd1 forms a heterodimer with Nab3 that likely binds cooperatively to most NNS terminators. Additionally, Nrd1 may recruit the Sen1 helicase to RNAP II to aid displacement of RNAP II from DNA.

Oxford Nanopore direct RNA sequencing (dRNA-seq) efficiently maps both mRNA cleavage and polyadenyation sites and NNS termination sites. No cleavage occurs in NNS termination but the 3' ends created by RNAP II are polyadenylated by Trf4/Pap2 in the TRAMP complex, which recruits the nuclear exosome for 3'-processing to remove the terminator sequences. The dRNA-seq adapter is ligated to the polyA tail, selecting for adenylated transcripts in whole cell RNA preparations. We have used this method to interrogate the transcriptome-wide effects of substitutions in Hrp1, Nrd1, and the Rpb3 subunit of RNAP II.

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Widespread regulatory divergence but stable expression in interspecific yeast

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Evolutionary changes in gene regulation are a key driver of phenotypic diversification across species. Comparison of gene expression patterns between parental species and F1 hybrids provides a way to identify cis and trans effects on gene expression. We performed RNA-seq in *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, and an F1 hybrid of these two species. We find that approximately 40% of yeast genes show significant regulatory divergence. Cis-only and trans-only effects each account for ~11–12% of genes. For genes with both cis and trans effects, most are compensatory such that the majority of yeast genes (81.5%) are maintained within the parental expression range. To link cis divergence to changes in the promoter sequence, we dissected the strongly cis-diverged locus *LYS2*. *S. cerevisiae* has a stronger Fkh2-like motif and carries a 9-bp AT-rich insertion and an additional proximal TATA-like element that are absent from *S. paradoxus*. This derived promoter-architecture is predicted to reshape local chromatin, leading to nucleosome depletion in *S. cerevisiae* but higher occupancy in *S. paradoxus*. In addition, we extended our study to test whether post-transcriptional regulation is similarly robust in hybrids, we quantified allele-resolved intron retention. We find that intron retention is broadly conserved between the species with only five allele-specific differences. Together, widespread compensatory cis–trans evolution contrasts with rare, locus-specific splicing changes in the hybrid, supporting robust regulatory control in interspecific yeast hybrids

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Comprehensive mapping of mitochondrial tRNA modifications and modeling of a mitochondrial disease-associated mutation in an RNA modifying enzyme

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Saccharomyces cerevisiae is an invaluable model in the study of mitochondrial tRNA biology. Yet the positions of modified bases in all yeast mitochondrially-encoded tRNAs (mt-tRNAs) are still not fully mapped. We performed Nanopore direct RNA sequencing (DRS) on tRNAs from the crude mitochondrial fraction of yeast to map base modifications across all 24 mt-tRNA isoacceptors. Additionally, we developed a method to detect dihydrouridine (D) sites in tRNAs, tD-seq, where chemical reduction of dihydrouridine causes disruptions to reverse transcription. We mapped dihydrouridine, pseudouridine, and N2-dimethylguanosine sites in mt-tRNAs using DRS, tD-seq, and knockouts of five conserved tRNA-modifying enzymes. Our results establish Dus1 and Dus2 as the enzymes responsible for D_{14}, D_{16}, D_{17}, D_{17a}, and D_{20} formation in *S. cerevisiae* mt-tRNAs, and revealed interactions between Dus1, Dus2, and Trm1-catalyzed modifications. We provide a comprehensive analysis of *S. cerevisiae* mt-tRNA base modifications, and identify novel modification "circuits" in yeast mt-tRNAs, in which the loss of a single enzyme's activity can change modification levels at sites catalyzed by other enzymes. These findings expand our understanding of mt-tRNA base modifications and their interdependence, and advance opportunities for the yeast model for investigating defects in human mitochondrial function resulting from dysregulation of RNA modifications. To this end, we used budding yeast to model a mutation in the human tRNA/mRNA pseudouridine synthase gene PUS1 that is associated with the mitochondrial disease MLASA. We found that this mutation at the orthologous site in yeast *PUS1* causes a respiratory growth defect. Curiously, the yeast Pus1 enzyme does not modify mt-tRNAs and is not localized to yeast mitochondria, but rather to the nucleus. We are investigating how this disease-associated mutation impacts Pus1 activity in yeast, and which activities of nuclear Pus1 critically influence yeast mitochondrial function.

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Too much of a good thing: How α-arrestins help cells survive amino acid overload

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Excess amino acids cause cellular cytotoxicity. α-Arrestins are protein trafficking adaptors that facilitate amino acid transporter (AAT) internalization, controlling intracellular amino acid levels. When α-arrestins are absent, AATs remain at the cell surface, potentially causing excessive amino acid accumulation. While α-arrestin-mediated AAT regulation is well-documented, their physiological role in preventing amino acid overload remains largely unknown. Using *Saccharomyces cerevisiae*, we explored loss of α-arrestin Art1 (*art1*Δ) as a model for dysregulated amino acid homeostasis.

Metabolomics revealed that *art1*Δ cells accumulate elevated arginine and lysine (Arg/Lys). This excess reduces mitochondrial membrane potential and impairs respiration. Further, *art1*Δ cells exhibit enlarged, hyper-acidic vacuoles with altered proteome and lipid composition. Since mitochondria are the major site of arginine degradation and vacuoles store over 90% of cellular basic amino acids, organelle dysfunction likely stems from Arg/Lys overload.

What pathways manage this excess? RNAseq analysis revealed strong induction of: (1) the arginine degradation pathway and (2) the phosphate regulon. Arginine degradation reduces accumulation by converting arginine into other metabolites. The phosphate regulon enhances polyphosphate synthesis, increasing vacuolar arginine storage capacity. Interestingly, both pathways depend on *ARG82*, a transcriptional regulator of arginine metabolism that moonlights as an inositol phosphate multikinase, the product of which activates polyphosphate synthesis. Despite the role of increased polyphosphate synthesis in augmenting basic amino acid storage in vacuoles, this pathway is dispensable for *art1*Δ survival, while arginine degradation is critical.

In conclusion, increased Arg/Lys uptake in *art1*Δ cells causes vacuolar and mitochondrial dysfunction, clarifying the relationship between cellular metabolism and organelle function. These conserved pathways make our findings relevant to systemic dysfunctions and metabolic diseases.

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Dynamic Reciprocal HXK2-PGI1 and HXK2-G6PDH Multienzyme Complexes Formation is associated with flux changes in Yeast metabolism

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Metabolic homeostasis is contingent upon the precise partitioning of carbon flux at pivotal pathway decision nodes (branch points in metabolic pathways). While transient multienzyme assemblies, or metabolons, are recognized for facilitating substrate channeling and enhancing catalytic throughput, their function as high-level regulatory switches at metabolic pathway branches remains largely enigmatic. This study investigates the hypothesis that the reciprocal, dynamic association of adjacent enzymes at metabolic pathway branches serves as a fundamental mechanism to fine-tune flux distribution between interconnected pathways, using the conserved glycolysis - oxidative pentose phosphate pathway (OPPP) metabolic pathway branch in *Saccharomyces cerevisiae* as a model system.

To interrogate these interactions without inducing regulatory artefacts, we implemented scarless genomic tagging coupled with NanoBiT split-luciferase complementation. Utilizing a robust suite of *in vitro* and *in vivo* protein-protein interaction (PPI) assays, we characterized the interaction of yeast hexokinase (HXK2) with its divergent downstream partners: phosphoglucose isomerase (PGI1, glycolysis) and glucose-6-phosphate dehydrogenase (G6PDH, OPPP).

Our findings reveal that HXK2 forms distinct, mutually exclusive complexes that directly modulate the kinetic potency of its partners. Specifically, the HXK2-PGI1 association significantly enhances PGI1 activity, promoting glycolytic flux progression. Conversely, HXK2-G6PDH complex formation upregulates

G6PDH activity, potentially shifting flux toward the OPPP. Notably, the association-dissociation kinetics of these pairs are sensitive to fluctuations in cellular metabolic intermediates, respiratory flux, and redox states, indicating a sophisticated feedback/feedforward regulatory loop.

These results demonstrate that the association of HXK2 with PGI1 or G6PDH is modulated by metabolic conditions regulating glycolysis – OPPP flux distribution. Altogether, our findings suggest a model where HXK2 acts as a metabolic sensor, undergoing reciprocal assembly with PGI1 or G6PDH to form dynamic multienzyme complexes that may function as regulatory switches playing a key role in reconfiguring flux distribution between glycolysis and OPPP by regulating enzyme activities.

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Proteome Dynamics in Desiccation and Rehydration

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The growing impact of climate change has heightened the urgency to understand how organisms adapt to environmental stresses, such as fluctuating water availability. Anhydrobiotes—organisms capable of surviving extreme desiccation—enter a state of stasis during water loss and resume normal metabolic functions upon rehydration. In recent years, the molecular mechanisms underlying this survival strategy have started to be revealed. However, this study represents the first effort to investigate how the majority of an organism's proteome is restructured in response to desiccation and rehydration. By leveraging the Yeast GFP-Fusion Collection, which covers 75% of the Saccharomyces cerevisiae proteome, we can monitor the localization and expression of 4,156 proteins during these stress conditions. A pilot study involving 20 GFP-tagged strains has already identified three distinct patterns of protein behavior following desiccation and rehydration: 1) proteins with unchanged localization, 2) delocalized or degraded proteins, and 3) proteins forming puncta. Expanding on this initial finding, we have employed automated microscopy and custom machine-learning algorithms to analyze thousands of yeast strains. We developed models to predict GFP expression patterns based on annotated ORFs and data from single-cell data connected to 19 localization classes. The screen revealed changes in protein abundance and a redistribution of proteins to the endosome and bud neck. Together, these findings highlight both the stability of the yeast proteome and its remodeling during desiccation and rehydration. A complete analysis of the yeast GFP-fusion collection will enable us to create a comprehensive map of proteome dynamics, providing insight to the protective mechanisms enabling anhydrobiosis.

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Cell Wall Integrity pathway maintains cell size, cytoplasmic concentration and cell fate

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Cells grow until they reach a critical size, at which point they divide. The cytoplasm is a complex and highly crowded environment that plays essential roles in intracellular processes; therefore, maintenance of cell size is critical for cell viability and cell fate. Recent studies have shown that excessive cell growth leads to cytoplasmic dilution, contributing to cellular dysfunction, irreversible cell-cycle arrest, and cellular senescence. However, the molecular mechanisms that sense cell size and regulate biophysical properties during excessive growth remain poorly understood. Here, we demonstrate a crucial role for the cell wall integrity (CWI) signaling pathway in these processes. Phosphoproteomic analysis indicated that the key CWI kinase Mpk1 is activated phosphorylating the downstream transcription factor Rlm1. Live-cell imaging showed that Mpk1 accumulates in the nucleus as cells enlarge, supporting the idea that Mpk1 becomes activated to promote Rlm1-dependent transcription during excessive cell growth. Deletion of *MPK1* further increased cell size in enlarged cells and diluted the cytoplasmic concentration. We measured cell wall thickness by transmission electron microscopy and found that Mpk1 is required to maintain proper cell wall thickness. Mathematical modeling based on these data suggested that Mpk1 is required to preserve cell wall resistance and balance turgor pressure as cell size increases, consistent with Laplace's law. Supporting this model, enlarged cells lacking Mpk1 were hypersensitive to mechanical and cell wall stress, including sonication and zymolyase treatment. Together, our results suggest that the CWI signaling pathway contributes to the regulation of cell size homeostasis and cytoplasmic biophysical properties. We propose a model in which a cell wall-based sensing mechanism balances turgor pressure, surface tension, and cytoplasmic crowding during excessive growth in a manner consistent with Laplace's law.

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Evolutionary rewiring of a conserved cell cycle pathway between *Saccharomyces cerevisiae* and the fungal pathogen *Cryptococcus neoformans*.

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Eukaryotic cells must spatially and temporally coordinate cytokinesis with nuclear division in order to maintain genome integrity. Budding yeasts provide a superb model to study this coordination because they designate the site of cytokinesis (division plane) at the bud neck before nuclear division (mitosis) begins. This spatial constraint requires machinaries to properly orient the mitotic spindle along the mother-bud axis and surveillance mechanisms to ensure correct genome inheritance in mother and daughter cells. In the model budding yeast *Saccharomyces cerevisiae*, a surveillance mechanism called the Spindle Position Checkpoint (SPoC) monitors spindle position and regulates exit from mitosis (cell cycle transition from M phase to G1) accordingly through the Mitotic Exit Network (MEN). It is unclear how conserved these mechanisms and pathways are in other fungi or budding yeasts. Here, we examined *Cryptococcus neoformans*, a critical priority fungal pathogen designated by the World Health Organization, to understand its strategies in safeguarding genome integrity during mitosis. While both divide by budding, *C. neoformans* diverged from *S. cerevisiae* more than 600 million years ago. Characterization of *C. neoformans* MEN components and pathway function have revealed a fascinating case of pathway rewiring and changed function: while the MEN regulates mitotic exit in *S. cerevisiae*, we found that the *C. neoformans* MEN controls cytokinesis. Strikingly, we also found that the key upstream signal which regulates the activation of MEN in *S. cerevisiae*, spindle position, does not regulate cell cycle progression in *C. neoformans*. In essence, there appears to be no SPoC in this organism. Our results revealed that both the input and output of a conserved cell cycle pathway have been rewired between *S. cerevisiae* and *C. neoformans* with implications on the evolution of cell cycle control and genome integrity maintenance.

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Self-propelled collective migration in yeast Megan M Halfmann[1], Randal Halfmann[1,2], Justin Mehojah[1], Boris Rubinstein[1], Jeffrey J Lange[1], Ameya Mashruwala[3] [1]Stowers Institute for Medical Research, [2]Biochemistry and Molecular Biology, University of Kansas Medical Center, [3]Mashruwala Lab, Stowers Institute for Medical Research

Colonies of *Saccharomyces cerevisiae* expanding on nutrient agar typically grow isotropically, producing dense circular colonies without active surface migration. Here we report a striking alternative growth mode in which colonies generate coordinated outward movement of multicellular groups, producing dynamic and self-avoiding projections across the agar surface. The motility phenotype requires simultaneous loss of the fatty acyl-CoA synthetases Faa1 and Faa4, previously shown to promote long-chain fatty acid activation, and is independent of surface adhesins that are required for all other multicellular phenomena in yeast. Motile colonies displayed extensive iridescent crystalline deposits and a prominent fluid halo surrounding the colony perimeter. We hypothesized that secretion of amphipathic lipid metabolites generates a surface tension gradient that drives outward collective movement via Marangoni flow. Mass spectrometric analysis of halo fluid identified 12-carbon lysophosphatidylcholine (C12 LPC) as a candidate surfactant. Remarkably, exogenous application of C12 LPC to nascent wild-type colonies sufficed to drive anisotropic multicellular outward flow, phenocopying the *faa1Δ faa4Δ* mutant. Ongoing genetic and biochemical analyses, including a genome-wide screen of the yeast deletion collection, have uncovered candidate modifiers of lipid metabolism and cell surface properties required for motility. Together, our findings reveal that small genetic changes acting through simple physical principles can produce complex multicellular behaviors in a prototypical unicellular organism.

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The genetic basis of hybrid fitness in fermentation environments Antoine Houtain, Nathan Brandt, Caiti Smukowski Heil North Carolina State University

Hybridization between different species can result in increased fitness in hybrid offspring, known as heterosis or hybrid vigor. Yeast hybrids have been isolated from a range of environments, but most notably, hybrids have been repeatedly sampled from wine, beer, and cider fermentations. Whether this reflects sampling bias, increased opportunity for hybridization in human associated environments, or a benefit of hybridization in these environments has been challenging to untangle, in part because hybrid spore viability is highly reduced making genetic dissection of traits impossible. Here, we suppress expression of genes *SGS1* and *MSH2* during meiosis to rescue hybrid fertility and generate recombinant hybrid haploids of *Saccharomyces eubayanus* and *Saccharomyces uvarum*. These sister species are found in various fermentations as complex hybrids (like *S. bayanus*) and as strains with introgression. We recovered meiotic products of 50 meioses (200 recombinant haploids), and measured growth phenotypes in nine fermentation relevant environments including different carbon sources, temperatures, and ethanol content. We identified quantitative trait loci (QTL) associated with ethanol content and growth in apple juice, and a pleiotropic QTL in which the *S. eubayanus* allele on the telomeric end of chromosome 4 is beneficial to growth in most environments. We backcrossed the haploid collection to each parent, creating two diploid sets in which regions of the genome are heterozygous or homozygous for parental ancestry. We phenotyped these backcrossed diploids in 5 environments (maltose, fructose, ethanol content, and cold temperature), allowing us to map loci underlying increased fitness in hybrids. Our work furthermore allows us to explore fitness effects of loss of heterozygosity events across the genome, which have previously been implicated in increased hybrid fitness. Overall, our results shine light on why hybrids are found in particular environments.

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Massively parallel interrogation of the fitness of natural variants in ancient signaling pathways reveals pervasive local adaptation Jose Aguilar-Rodriguez[1,2], Jean C. C. Vila[1], Shi-An A. Chen[1,3], Manuel Razo-Mejia[1,3], Olivia M. Ghosh[1,4], Daniel F. Jarosz[2,5], Hunter B. Fraser[2], Dmitri A. Petrov[1,6] [1]Department of Biology, Stanford University, [2]Department of Chemical and Systems Biology, Stanford University School of Medicine, [3]Present address: Altos Labs, Bay Area Institute of Science, [4]Department of Physics, Stanford University, [5]Department of Developmental Biology, Stanford University School of Medicine, [6]Chan Zuckerberg Biohub

The nature of standing genetic variation remains a central debate in population genetics, with differing perspectives on whether common variants are almost always neutral as suggested by neutral and nearly neutral theories or whether they can commonly have large functional and fitness effects as proposed by the balance theory. We address this question by mapping the fitness effects of over 9,000 natural variants in the Ras/PKA and TOR/Sch9 pathways—key regulators of cell proliferation in eukaryotes—across four conditions in *Saccharomyces cerevisiae*. While most variants are neutral in our assay, ~3,500 exhibited significant fitness effects. These non-neutral variants tend to be missense and to affect conserved, more densely packed, and less solvent-exposed protein regions. While some of these non-neutral variants are younger and rarer, and more often found in heterozygous states—consistent with purifying selection—a substantial fraction is present at high frequencies in the population, which is expected under balancing selection. Indeed, we find that variants with a positive fitness effect in our laboratory measurement show strong signs of local adaptation as they tend to be found specifically in domesticated strains isolated from human-made environments. Our findings support the view that while many common variants might be effectively neutral, a significant proportion have locally adaptive functional consequences and are driven into a subset of the population by local positive selection. This study highlights the potential to combine high-throughput precision genome editing with fitness measurements to explore natural genetic variation on a pathway-wide scale, thereby bridging the gap between population genetics and functional genomics to understand the nature of evolutionary forces in the wild.

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Adaptive variation in mutagenesis driven by protein self-assembly Alexandria Van Elgort[1], Christopher Jakobson[1], Yiwen Chen[1], James Byers[1], Raymond Futia[2], Thomas Lozanoski[3], Zachary Harvey[1], Jinglin Xie[1], David Garcia[1], Daniel Jarosz[1,4] [1]Chemical and Systems Biology, Stanford University, [2]Biology, Stanford University, [3]Bioengineering, Stanford University, [4]Developmental Biology, Stanford University

Mutations supply the raw material for evolution. However, most are not adaptive. Thus, hypermutation is typically transient. Nonetheless, modeling predicts that heritable but reversible 'mutagenesis switches' would provide strong advantages in many selective landscapes. Here we report that a frequent prionic switching capacity in DNA repair and recombination proteins provides such a mechanism across evolving *Saccharomyces cerevisiae* populations from the laboratory, nature, and the clinic. Self-templating transforms the functions and interactions of multiple DNA fidelity factors, altering adaptation while simultaneously providing resilience to genotoxic stress. In the WHO priority pathogen *Candida albicans*, 300 million years diverged from *S. cerevisiae*, prion inheritance factors accelerate evolution of fluconazole resistance, compromising the mainline therapy for this organism. Our findings demonstrate that protein self-assembly, a collective molecular behavior common among DNA replication and repair factors, can materialize epigenetic memory that tunes rates of genome diversification to expand adaptive opportunities.

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From retrotransposon to centromere: Ancient co-option and contemporary centromere birth

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Centromeres ensure faithful chromosome segregation by assembling the megadalton kinetochore complex that couples centromeric DNA to spindle microtubules. While kinetochore components are broadly conserved across eukaryotes, centromere DNA evolves rapidly—raising the question of how new centromere architectures arise. The yeast point centromere exemplifies this challenge: how did sequence-defined centromeres evolve from ancestral repeat-based, epigenetically specified forms? We find that the LTR retrotransposon Ty5 has had an outsized impact on budding yeast centromere evolution, seeding a newly discovered "proto-point" centromere and its evolutionarily related point centromere. Ongoing comparative work further suggests a contemporary, Ty5-associated instance of sequence-defined centromere evolution. These results suggest repeated emergence of sequence-defined centromeres in the budding yeast clade and implicate Ty5 in driving this sequence evolution, highlighting an underappreciated interplay between chromosome segregation machinery and mobile genetic elements in yeasts.

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Single-cell eQTL Mapping Reveals Environment-dependent Genetic Regulation

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Expression quantitative trait locus (eQTL) mapping identifies genetic variants that regulate gene expression and contribute to phenotypic diversity. eQTL studies in controlled crosses have revealed the genetic architecture of natural variation in gene expression within specific genetic backgrounds. More recently, population-based eQTL studies have identified genetic variants in diverse *S. cerevisiae* strains isolated from various ecological niches. However, how genetic variants influence gene expression across different environments within a natural population remains unexplored. Traditionally, bulk RNA-seq is used to identify eQTLs, but it averages expression across entire populations, losing cellular heterogeneity and cell state information. To address these limitations, we developed a multiplexed single-cell RNA sequencing (scRNA-seq) approach to map eQTLs across ~ 96 genetically diverse *S. cerevisiae* strains under multiple conditions including rich media, fluconazole treatment, and synthetic rich media.

We used scRNA-seq for simultaneous cell genotyping, gene expression measurements, and cell cycle phase classification. We mapped eQTLs to SNPs, indels, and structural variants (SVs) using linear mixed modeling to comprehensively assess genetic effects across variant types, as well as stratified by cell cycle phase. We identified both local and distal eQTLs, with more trans-regulatory eQTLs across conditions. Notably, SVs exhibited particularly strong trans-regulatory effects, suggesting that SVs potentially drive broad regulatory changes. Trans-eQTLs exhibited phase-specific patterns, with non-overlapping sets of trans-regulatory loci in G1, S, and G2/M phases. Comparing rich media to fluconazole-treated conditions revealed that many of the eQTLs are environment-specific in these two environments, likely reflecting the distinct physiological demands of nutrient-rich versus stress conditions. These findings demonstrate that natural genetic variation shapes gene expression in environment-dependent ways. Thus, providing insights relevant to understanding phenotypic diversity in natural populations.

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One-trick versus keystone gene families: genomic and metabolic lessons from more than 400 million years of yeast evolution

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How yeasts have evolved and functionally encode their diverse metabolisms has been a major question in yeast genetics, ecology, and evolution. The subphylum Saccharomycotina includes the model budding yeast *Saccharomyces cerevisiae* and has radiated into more than 1,000 known species over the course of more than 400 million years. The Y1000+ Project (http://y1000plus.org) recently published the genomes, high-throughput phenotypic data with an emphasis on carbon source utilization, and a genotype-phenotype map for essentially every known yeast species. These data revealed pervasive gene and trait loss, but also gains and regains, suggesting frequent independent evolutionary transitions and multiple evolutionary dynamics at work. Here, we will highlight recent discoveries with a focus on two different types of gene families that have evolved by distinct mechanisms to control yeast metabolisms: one-trick gene families that control a single carbon utilization trait and keystone gene families that affect multiple carbon utilization traits. The most influential keystone gene family encodes alpha-glucoside transporters, which affect the utilization of *MAL*tose and a dozen other alpha-glucosides. The genomes of some species are predicted to encode dozens of alpha-glucoside transporters, suggesting a highly dynamic and complex evolutionary history. We will highlight the classic Leloir *GAL*actose utilization pathway as a one-trick gene family that has frequently been lost during evolution and occasionally regained by horizontal gene transfer. Even for galactose metabolism, mysteries remain because we show how some yeasts possess an alternative *altGAL* pathway that instead uses multiple oxidoreductive reactions to catabolize galactose. The *altGAL* pathway may both blur the lines between one-trick and keystone gene families and suggest how transitions between these modes occur during evolution.

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The CellMap 2.0: An integrated model of yeast gene function

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We developed and applied a computational pipeline to integrate over 100 gene and protein interaction networks linking functionally related genes in the yeast *Saccharomyces cerevisiae*. The resulting gene embeddings form the CellMap 2.0, a comprehensive schematic of yeast gene functional interactions that covers ~98.5% of coding genes. It captures protein complex, pathway and bioprocess membership with greater fidelity than any individual network alone and outperforms existing integrated gene function models. The CellMap 2.0 improves the original genetic interaction-based CellMap 1.0 in terms of gene coverage and the quality and quantity of gene function annotations. Included in the CellMap 2.0 are literature networks we constructed through text mining over 3 million gene mentions from ~82,000 full-text articles and abstracts, which were subject to large language model summarization and embedding, as well as embedding human-generated gene function descriptions derived from SGD. We performed an interpretability analysis to determine the contribution of individual datasets, technologies and assay types to the CellMap 2.0 gene embeddings and corresponding functional performance, which provides a perspective on the quality, coverage and heterogeneity of yeast gene function experiments. Through ortholog mapping, we used the CellMap 2.0 to predict functional interactions for ~2,400 human genes and outperformed predictions obtained from integrating human datasets. Finally, we used the CellMap 2.0 to assign functions for ~20% of uncharacterized yeast genes.

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Taming wild genetic variation to learn a proteomic blueprint of the yeast cell

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As the primary functional units of the cell, proteins are a key bridge from changes in the genome to their consequences for cells and organisms. Yet most genetic variants are of unknown function, in part because the majority of proteins have been inaccessible to high-throughput measurements – even in model organisms. Here, building on the latest advances in mass spectrometry-based proteomics, we acquired exceptionally deep proteomes of nearly 1,000 closely related progeny of two wild budding yeast isolates. This natural atlas of more than 5,000 proteins encompassed over 80% of all predicted protein-coding genes in *Saccharomyces cerevisiae*, including 95% of essential genes. Protein-protein covariation driven by natural genetic diversity revealed detailed molecular relationships from individual complexes to metabolic pathways to organelles. Natural hypomorphic alleles disrupted these functional profiles, and super-resolution genetic mapping identified tens of thousands of mutation-protein connections controlling protein abundance. The unprecedented coverage we obtained across low-abundance proteins (such as transcription factors and other regulators) allowed us to pinpoint the *cis*-acting mechanisms that drove proteome remodeling in *trans*, including synonymous mutations. Tracing these molecular connections revealed how variants throughout the genome drove complex traits, such as azole resistance governed by master regulators like *PDR1*. Together, our results illustrate how ultra-deep proteomics can comprehensively describe the molecular architecture, genetic control, and phenotypic consequences of natural proteomic diversity.

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Conditional genetic interaction analysis uncovers the metabolic landscape of WGD paralogs

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Whole-genome duplication (WGD) events have played a pivotal role in shaping the genomes of organisms across the tree of life. In the ancestor of *Saccharomyces cerevisiae,* WGD occurred ~100 million years ago, which coincided with the emergence of angiosperms. Since then most paralogs have been eliminated and the factors influencing the maintenance of the persisting paralogs remain poorly understood. Here, we used trigenic Synthetic Genetic Array (t-SGA) to conduct a conditional fitness analysis of 79 double and corresponding 158 single gene deletion mutants of WGD paralogs, which have previously been shown to exhibit sparse digenic and trigenic interaction profiles under standard nutrient-rich growth conditions. We profiled the mutants across a range of metabolic environmental conditions comprising 30 combinations of six carbon (glucose, galactose, maltose, raffinose, sucrose and fructose) and five nitrogen (glutamate, glutamine, proline, urea and allantoin) sources. 80% of single mutants showed a 5% growth defect and 30% of double mutants showed a negative digenic interaction under at least one metabolic condition suggesting that these paralogs are important for growth in different carbon and nitrogen sources. The greatest number of single mutants exhibited growth defects when grown in galactose and maltose suggesting specialization in ripening and metabolic shifts during fruit maturation. Meanwhile, double mutants showed negative interactions on conditions containing fructose suggesting these paralogs were selected for a buffering capacity in a high-sugar environment of fruit. On average paralogs were more likely to be carbon specialists and nitrogen generalists. Additionally, we observed that sequence variation in these paralogs was associated with growth of natural isolates in different carbon sources suggesting that these paralogs may be important for their ecological niche. This study provides insight into the evolutionary forces that shape genomes.

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The proportional scaling of mRNA and ribosome concentrations controls eukaryotic cell growth

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Cell growth underlies nearly all eukaryotic physiology, yet its quantitative principles remain unclear. Using single-molecule ribosome tracking, spike-in RNA sequencing, and quantitative proteomics across 15 nutrient-limited conditions in budding yeast, we define how growth is controlled in the budding yeast *Saccharomyces cerevisiae*. Ribosome concentration scales linearly with growth rate, while peptide elongation speed remains constant at ~9 amino acids s^{-1}, implying elongation is not a regulatory lever. Instead, total mRNA concentration increases proportionally with ribosomes to accelerate growth. A simple kinetic model of mRNA–ribosome binding accurately predicts the fraction of active ribosomes, growth rate, and responses to transcriptional or size perturbations. Consistent with this model, transient inhibition of mRNA degradation boosts growth by elevating mRNA concentration. These results reveal that eukaryotic cells accelerate proliferation primarily by proportionally scaling mRNA and ribosome abundance, establishing a quantitative framework for understanding eukaryotic biosynthesis.

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The molecular determinants of Ty1 retrotransposon restriction specificity in budding yeast

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The evolutionary history of retrotransposons and their hosts shapes the dynamics of transposition and restriction. The *Pseudoviridae* of yeast includes multiple Ty1 LTR-retrotransposon subfamilies. *Saccharomyces cerevisiae* prevents uncontrolled retrotransposition of Ty1 subfamilies using distinct mechanisms: canonical Ty1 is inhibited by a self-encoded restriction factor, p22/p18, whereas Ty1' is inhibited by an endogenized restriction factor, Drt2. The minimal inhibitory fragment of both restriction factors (p18m and Drt2m) is a conserved C-terminal capsid domain. Here, we use biophysical and genetic approaches to demonstrate that p18m and Drt2m are highly specific to their subfamilies. Although the crystal structures of p18m and Drt2m are similar, three divergent residues found in a conserved hydrophobic interface direct restriction specificity. By mutating these three residues, we re-target each restriction factor to the opposite transposon. Our work highlights how a common lattice-poisoning mechanism of restriction evolved from independent evolutionary trajectories in closely related retrotransposon subfamilies. These data raise the possibility that similar capsid-capsid interactions may exist in other transposons/viruses and that highly specific inhibitors could be engineered to target capsid interfaces.

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Systematic genetic characterization of the human PKR kinase domain highlights its functional malleability to escape viral pseudosubstrate mimics

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Evolutionary arms races can arise at the contact surfaces between host and viral proteins, producing dynamic spaces in which genetic variants are continually pursued. However, the sampling of genetic variation must be balanced with the need to maintain protein function. A striking case is given by the human innate immune protein kinase R (PKR). PKR detects viral replication within the host cell and halts protein synthesis to prevent viral replication by phosphorylating eIF2α, a component of the translation machinery. PKR is targeted by many viral antagonists, including poxvirus pseudosubstrate antagonists that mimic eIF2α. Remarkably, PKR has rapidly evolving residues at the eIF2α-binding interface, suggesting it is engaging in an evolutionary arms race, despite the surface's critical role in phosphorylating eIF2α.

Yeast provides a fantastic system to systematically characterize the evolutionary opportunities available at this dynamic interface: despite PKR being nonnative to yeast, expressing it still leads to eIF2α phosphorylation and growth arrest, which can be reversed by co-expression of viral PKR antagonists. Thus, yeast growth can be used as a readout of the function of PKR variants against its antagonists. We fist generated and characterized a library of 426 SNP-accessible variants of human PKR for their ability to escape inhibition by the model pseudosubstrate inhibitor K3 from vaccinia virus. We found K3-resistant variants are readily available throughout the interface and are enriched at sites under positive selection. We have extended our screen of PKR variants to test them against viral pseudosubstrate inhibitors from the poxviruses variola and tanapox, which vary in their homology to vaccinia virus K3 and their strength of PKR inhibition. We find that many PKR variants that evade vaccinia K3 also evade the variola and tanapox inhibitors. Moreover, we find that many of the beneficial variants were also beneficial against the independently arisen pseudosubstrate inhibitor vIF2α from Ranavirus. Overall, we find that the eIF2α-binding surface of PKR is highly malleable and that beneficial variants against one virus often also provide benefits against other viruses. Together, these effects potentiate PKR's evolutionary ability to combat viral inhibition.

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The yeast RECQL4 homolog Hrq1 is a novel RNA polymerase III inhibitor

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Mutations in the human RECQL4 helicase cause three autosomal recessive disorders and are strongly associated with cancer predisposition, yet the molecular basis of disease remains poorly understood. The *Saccharomyces cerevisiae* RECQL4 homolog Hrq1 has established roles in DNA interstrand crosslink repair and telomere maintenance. Here, we uncover an unexpected function for Hrq1 in the regulation of RNA polymerase III (RNAP3) transcription. To define Hrq1's genomic sites of action, we developed HELI-PAD (Helicase Engagement Linked to Induced Polymorphisms After Deamination), a genome-wide mapping approach for helicase localization. Unexpectedly, Hrq1 localized to the transcription start site and ~25 bp downstream of the termination site of all RNAP3-transcribed genes. Mass spectrometry revealed that Hrq1 physically associates with 8 of the 17 RNAP3 subunits. Consistently, synthetic genetic array analysis identified genetic interactions between *hrq1Δ* or the catalytically inactive *hrq1-K318A* allele and mutations in genes encoding RNAP3 subunits, transcription factors, and nutrient-sensing regulators. Although loss of *HRQ1* does not impair fitness under

standard growth conditions, *hrq1* mutants are sensitive to RNAP3 stressors, phenocopying deletion of the canonical RNAP3 inhibitor Maf1. Quantitative RT-PCR demonstrated that pre-tRNA levels increase in *hrq1Δ* cells, whereas Hrq1 overexpression suppressed pre-tRNA accumulation. Together, these findings establish Hrq1 as a negative regulator of RNAP III transcription. These data reveal a previously unrecognized role for a RecQ4-family helicase in transcriptional regulation and suggest that dysregulated RNAP3 activity may contribute to RECQL4-associated disease. Ongoing studies are investigating distinct catalytic and structural roles of Hrq1 at RNAP III genes, its function in tRNA gene-mediated silencing, and its potential cooperation with the Sen1 helicase in R-loop resolution.

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Elevated and skewed dNTP pools alters replication fork progression and Okazaki fragment processing

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The fidelity of DNA replication ensures the stability of the genome from cell division to the next. Accurate DNA synthesis by replicative DNA requires appropriate levels and ratios of deoxyribonucleotide triphosphates (dNTPs), the building blocks of DNA. dNTP pools are tightly regulated and rely on ribonucleotide reductase (Rnr) to catalyze the rate limiting step in dNTP synthesis. Regulatory mutations in *RNR1* lead to defined changes in dNTP pools. When dNTP pools are dysregulated, it interferes with the normal functioning of the replisome. Elevated dNTP pools lead to an increased rate of nucleotide misincorporation, increase the rate of DNA replication and alter the number of replication origins that are activated. We previously used deep sequencing to demonstrate that altering the dNTP pools in different ways (elevated, skewed) leads to distinct mutation profiles. These misincorporation events are substrates for DNA mismatch repair (MMR), which acts as a spell-check for replicative DNA polymerases, but as the mutation rates increase, MMR can become saturated. Thus, altered dNTPs can promote mutagenesis that can lead to cancer. At the same time, cancer cells have elevated dNTP pools to maintain rapid proliferation. This can, in turn, lead to further mutagenesis and promote the molecular evolution of the cancer, providing a selective advantage.

We previously performed previous synthetic lethal (SGA) screens in *Saccharomyces cerevisiae* strains with *rnr1D57N, rnr1Y285F* and *rnr1Y285A,* mutations that elevate and/or skew the dNTP pools in distinct ways. In addition to almost all of the genes involved in MMR, we identified a number of genes whose products are involved in DNA replisome progression and Okazaki fragment processing. These results suggested that altered dNTP pools compromise progression of the replication fork. We have used Okazaki fragment sequencing to characterize the effect of altered dNTP pools on both Okazaki fragment processing and replisome progression in all three *rnr1* backgrounds. Analysis of the Okazaki fragments indicate clear differences in Okazaki fragment processing and replication fork directions, including potential changes in origin firing, when dNTP pools are altered. We are currently working to develop a mechanistic understanding of these observations. This work provides important insights into the ways in which elevated and skewed dNTPs compromise replisome functions. By understanding how these pathways are disrupted when dNTPs are altered, we have the potential to target these pathways for chemotherapeutic purposes.

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Spontaneous un-selected mutation patterns in *Candida albicans* reveal the relative stability of alternative ploidy states

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Spontaneous mutation generates not only allelic variation throughout the genome, but also variation in the architecture of the genome itself. The opportunistic human pathogen *Candida albicans* is a budding yeast with the potential to grow and evolve in several ploidy states, providing diverse avenues for adaptation. We characterized spontaneous rates of un-selected genetic change in this organism, both in terms of transitions between ploidy states and the generation of aneuploid karyotypes, as well as genome-wide rates of point mutation in each cell type. We conducted mutation accumulation in >200 lines derived from two genetic backgrounds, with over 700 generations per line. We detected over 1800 point mutations, numerous karyotypic changes, and loss-of-heterozygosity events. Diploids showed point mutation rates similar to other yeast species, few karyotypic changes, and subtle differences between genetic backgrounds. In tetraploids, which can be formed as part of a parasexual process of mating between diploids, we found relatively high point mutation rates, with a shift towards deletions, and frequent karyotypic changes. It is hypothesized that tetraploids undergo chromosome loss to return to diploidy without meiosis, but we find that a third of karyotypic changes in this cell type involved increases in chromosome copy number, and that the return to diploidy is a random mutational process. In haploids, which are believed to be a particularly unstable cell type, we observed frequent diploidization events, with the resulting homozygous diploids exhibiting aneuploidy and higher point mutation rates than heterozygous diploids. Mutations were generally deleterious in diploids, but were more likely to be beneficial in the other cell types. We find no evidence that spontaneous karyotypic variation in tetraploids provides resistance to the antifungal drug fluconazole. Our study reveals how the mutation process both responds and contributes to genomic variation in a highly evolvable human pathogen.

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Human Oncogenic Transcription Factor MITF Induces Centromeric Transcription and Adaptive Aneuploidy in *Saccharomyces cerevisiae*

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The transcription of centromeric DNA into non-coding RNAs (cenRNAs) is crucial for kinetochore assembly and faithful chromosome segregation. In budding yeast, this process is tightly regulated by the basic Helix-Loop-Helix (bHLH) transcription factor Cbf1. Despite its importance, the regulatory mechanisms linking cellular stress to centromeric transcription remains poorly understood. We hypothesized that stress-induced, conserved bHLH transcription factors, such as the human melanoma oncogene MITF, might orchestrate centromeric transcription to drive adaptive chromosomal instability.

To dissect the mechanistic consequences of MITF activity, we utilized *Saccharomyces cerevisiae* as a functional model. We identified a striking morphological phenotype and found that expression of human MITF dramatically increases overall cenRNA levels. By quantifying transcripts from every individual centromere, we discovered that Chromosome 3 exhibited the highest cenRNA upregulation. Underscoring a functional genetic interaction with the host machinery, we also identified that deletion of *CBF1* and mutant *CEP3* were highly sensitive to MITF expression.

Crucially, we explored the physiological consequences of this specific MITF-driven cenRNA spike. It is established that trisomy of Chromosome 3 in budding yeast confers tolerance to heat shock. Strikingly, wild-type yeast pre-conditioned with MITF overexpression exhibited significantly enhanced survival and robust growth following both acute and chronic heat shock compared to empty-vector control. While we also observe that DNA damaging agents and MITF perturbations alter cenRNA levels in human cancer cell lines, our yeast model provides a direct, functional readout of stress-induced adaptation.

These findings demonstrate that human MITF can interact with the yeast centromeric machinery to drive targeted cenRNA upregulation and specific, adaptive aneuploidy. This cross-species model provides a powerful platform to study how stress-induced centromeric transcriptional reprogramming fuels evolutionary adaptation and cancer cell fitness.

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Gene expression noise evolves more slowly and by different molecular mechanisms than gene expression in a model eukaryotic genus

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Gene expression is a stochastic process and varies among genetically identical cells in the same environment. This 'noise' in expression can be costly or beneficial depending on the context, and the relative prevalence of each context will determine how quickly differences in noise accumulate between species. The mechanistic coupling of noise to average expression raises the question of the extent to which they evolve independently or as a correlated unit, and if they evolve independently, through what mechanisms. Currently, however, the genome-wide rate of evolution and mechanisms underlying differences in noise are unknown, limiting our understanding of how gene regulation evolves. To address these questions, we used single-cell RNA-seq on two distantly related *Saccharomyces* yeast species. We found that noise was more similar between these species than was average expression, suggesting that noise is predominantly subject to purifying selection. In addition to the frequency of noise differences, the molecular basis of these differences reveals whether noise and average expression evolve through shared or distinct mechanisms. We therefore performed single-cell RNA-seq on the hybrid of the two species, using estimates of allele-specific expression in individual cells to identify *cis*- and *trans*-regulatory differences in expression noise. We found that *trans*-regulatory differences were more common than *cis*-regulatory differences for noise. By contrast, we observed the opposite pattern for average expression. Noise and average expression differences, therefore, likely arise from distinct molecular players. At the same time, we observed that expression of the two alleles in hybrid cells was often poorly correlated, suggesting independent control of the two alleles and that differences in noise are often attributable to intrinsic noise. The molecular players responsible for these intrinsic, but *trans*-regulatory, differences in noise are currently unknown. Overall, our results indicate that the evolution of noise is often decoupled from that of average expression, evolving at different rates and by different molecular mechanisms. This work thus provides a distinct window into the evolution of gene regulation that is not accessible through studies of differences in average expression alone.

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Noncanonical G-Quadruplex DNA Binds and Modulates Zinc Finger Transcription Factor in *Saccharomyces cerevisiae.*

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G-quadruplex (G4) DNA structures are non-canonical nucleic acid conformations that form within guanine-rich promoter regions and are increasingly recognized as influential regulatory elements. These higher-order structures can modulate transcriptional outcomes by altering protein–DNA interactions. Although several chromatin-associated proteins have been shown to bind G4 DNA, it remains unclear whether classical glucose-responsive transcription factors directly associate with these non-canonical structures. Using Saccharomyces cerevisiae as a model organism, we focused on Mig1, a zinc-finger transcription factor that mediates glucose repression. Our objective is to determine whether Mig1 function is influenced by the presence of G4 structures within gene promoters.

In this study, we investigated whether Mig1 directly associates G4 DNA using in-vitro and in-vivo approaches. Using EMSA, we showed that the recombinant, purified Zn finger domain of Mig1 specifically binds to G4-forming oligos. Additionally, oligo pull-down assays demonstrated the specific association of Mig1 with G4-forming sequences compared to non-G4 control sequences, supporting a direct interaction in vitro. To examine the association in a cellular context, we performed chromatin immunoprecipitation followed by qPCR (ChIP-qPCR) which revealed Mig1 occupancy at endogenous promoters predicted to form G4 structures, indicating that Mig1 associates with G4-containing loci in vivo. Furthermore, treatment with the G4-stabilising ligand PhenDC3 increased Mig1 promoter occupancy, demonstrating that G4 structural stabilization facilitates Mig1 transcription factor recruitment in a physiological chromatin context.

These results provide evidence that Mig1 binds G-quadruplex DNA both in vitro and in vivo, highlighting the relevance of non-canonical DNA structures for transcription factor targeting yeast. Together, these findings suggest that DNA secondary structure may contribute to glucose-responsive transcriptional regulation, offering new insight into the interplay between transcription factors and non-canonical DNA structures in gene regulation.

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Elp1 regulates heterochromatin independently of its canonical role in tRNA modification

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Heterochromatin is a repressive chromatin state that plays a central role in epigenetic gene regulation across eukaryotes, yet the mechanisms governing its formation remain incompletely understood. Using classical forward genetics in the fission yeast *Schizosaccharomyces pombe*, we recently identified Elongator Protein 1 (Elp1) as a novel regulator of heterochromatin [1]. Elp1 is best known as a conserved subunit of the Elongator complex, found in yeast, plants, and animals. Although Elongator was originally proposed to function in transcription and chromatin regulation, extensive work in the budding yeast *Saccharomyces cerevisiae* has led to the prevailing view that its primary role is to modify transfer RNAs (tRNAs), thereby broadly influencing translation and protein homeostasis [2]. In this framework, defects in Elongator are thought to cause pleiotropic phenotypes indirectly through loss of tRNA modifications. Consistent with this idea, Elongator destabilization in *S. cerevisiae* reduces heterochromatic gene silencing as a secondary consequence of tRNA defects [3]. Our most recent findings challenge this dogma by revealing, in *S. pombe,* that Elp1's role in heterochromatin is genetically separable from its canonical function in tRNA modification. We find that Elp1 cooperates with the RNA interference (RNAi) pathway and antagonizes another Elongator subunit, the putative lysine acetyltransferase Elp3, to promote heterochromatic gene silencing. We propose a new model in which Elp1 differentially regulates Elp3 activities in chromatin control versus tRNA modification, linking epigenetic regulation and translational control. Understanding how Elp1 contributes to chromatin regulation may provide new insights into Elp1-associated human disorders, including familial dysautonomia.

References:

1. Nirmal MB, Pearce ME, Liu CT, Finkel JM, Darrow KS, Vo TV. Deletion of Elongator Protein 1 (Elp1) relieves heterochromatin defects in a Pol II mutant of Schizosaccharomyces pombe. Genetics. 2026;232(2):iyaf258.

2. Dauden MI, Jaciuk M, Weis F, Lin T-Y, Kleindienst C, Abbassi NEH, Khatter H, Krutyhołowa R, Breunig KD, Kosinski J. Molecular basis of tRNA recognition by the Elongator complex. Science advances. 2019;5(7):eaaw2326.

3. Chen C, Huang B, Eliasson M, Rydén P, Byström AS. Elongator complex influences telomeric gene silencing and DNA damage response by its role in wobble uridine tRNA modification. PLoS genetics. 2011;7(9):e1002258.

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Kar4 acts as a Ste12 regulator in *Saccharomyces cerevisiae*, promoting Ste12 binding to a specific DNA motif genome-wide

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Transcription factors must often transcribe different genes under different conditions. To achieve this, DNA-binding specificity is often constrained by binding to cofactors or other transcription factors. Ste12 is a well-studied example in the budding yeast *Saccharomyces cerevisiae*. In addition to being the master transcriptional regulator of the yeast mating pheromone response, it also activates other transcriptional programs during asynchronous or filamentous growth. During the pheromone response, Ste12 is known to bind to a pair of pheromone response elements (PREs), although the orientation and spacing of PREs used by Ste12 in vivo has remained uncertain. In addition to Ste12, Kar4 is a putative transcription factor essential for mating. Kar4 has been shown to function with Ste12 to promote the transcription of a small subset of Ste12 targets required for efficient mating. However, the mechanism by which Kar4 modulates Ste12 activity has remained uncertain. Here, we examined the effect of Kar4 on Ste12's function at the levels of transcription (RNA-seq) and DNA binding (ChIP-exo). We show that during the pheromone response, Kar4 promotes Ste12 binding to nearly all Ste12 DNA-binding sites associated with transcriptionally-upregulated genes, even if their upregulation is not dependent on Kar4. We further found that the majority of Ste12-binding sites have a specific motif: two PREs separated by four nucleotides in a head-to-tail orientation (H-T 4 motif). Sites associated with Kar4-dependent transcription have PREs with more mismatches, and substantially lower Ste12 occupancy in *kar4*Δ cells, than sites linked to Kar4-independent transcription. This suggests that Kar4 is necessary for efficient binding of Ste12 to sub-optimal H-T 4 motifs. Interestingly, during the pheromone response in *kar4*Δ cells, Ste12 exhibits increased binding to non-H-T 4 motifs, particularly T-T 3 motifs, resulting in the abnormal upregulation of many genes not transcribed in wild-type. We propose that Kar4 functions by affecting the DNA-binding specificity of Ste12 globally, promoting its binding primarily to H-T 4 motifs, thereby targeting it to genes involved in the pheromone response.

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Repurposing Set1 to accommodate cellular response to nutrient depletion

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Cell fate determination requires faithful integration of extracellular signaling cues with internal genetic and epigenetic marks. Diploid cells of the budding yeast Saccharomyces cerevisiae divide mitotically when cultured in rich media but will undergo one of three partially overlapping cell responses when deprived of nutrition; filamentous growth, autophagy, or meiosis. Prior studies have highlighted the intricate balance established between gene expression programs and executing the correct cellular outcome, revealing a leading role for post-translational histone modifications. While much is known about how histone modifications control transcription, there is a gap in our knowledge regarding how histone modification machinery is repurposed to regulate cell fate decisions. Previous work in my laboratory has identified key genetic interactions between the Cdk8 Kinase Module subunits cyclin C-Cdk8 and the histone H3Lys4 methylation regulatory enzymes Set1 and Jhd2 that are at the center of the filamentation-autophagy-meiosis axis. We have found that CNC1 and JHD2 are required to inhibit filamentous growth in nutrient rich conditions and that Cdk8 antagonizes locus-specific H3Lys4 methylation during this process. Here, we provide further evidence that histone H3Lys4 methylation is redistributed in part through Set1 destabilization during meiosis. Using a chemical-genetic approach, we determined that meiotic Set1 degradation occurs independently of the meiotic transcriptional program and is instead regulated by the autophagy pathway. Since autophagy is required for meiosis, we next confirmed that Set1 destabilization is observed during autophagy.

In contrast to the Cdk8-dependent regulation of Set1 during meiosis, we found that its destruction during autophagy occurs independently of Cdk8. This suggests that there are distinct mechanisms that regulate Set1 turnover and H3Lys4 methylation as cells react to nutritional depletion and that these mechanisms may be critical determinants in the ultimate cellular response to stimuli. Finally, to identify the loci subject to Cdk8-mediated Set1 antagonism, we performed ChIP-seq experiments measuring histone H3Lys4 mono-, di-, or trimethylation levels in wild-type or cdk8 catalytically inactive mutants cultured in mitotic, autophagy, or meiotic growth conditions. These studies revealed nutrient-dependent H3 Lys4 methylation patterns and implicate Cdk8 in regulating key genes in the cell differentiation pathway.

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HMG-box protein Hmo1 inhibits transcriptional silencing in yeast cells and is antagonized by linker histone H1

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The HMG-box protein Hmo1 is an abundant chromatin protein in yeast, with diverse functions in chromatin compaction, genome stability, and transcription. Due in part to its atypically basic C-terminus, Hmo1 has been proposed to assume roles of linker histones in yeast cells. We examined the individual and combinatorial effects of Hmo1 and linker histone H1 on chromatin-mediated process in yeast, including Sir-dependent heterochromatin formation, chromosome segregation, recombination, and transcription. We find that cells lacking Hmo1 exhibit increased silencing of a reporter gene at *HMR*, while deletion of Hmo1's basic C-terminus has no effect on silencing. Unexpectedly, the increase in silencing caused by deletion of *HMO1* is abolished in strains also lacking histone H1. Similarly, cells lacking Hmo1 protein are defective in maintaining plasmid minichromosomes, but this defect is substantially suppressed by loss of histone H1. RNA-seq experiments on single and double knockout strains indicate that histone H1 does not substantially influence the transcription of genes that depend on Hmo1 for normal expression. Our results indicate that Hmo1 and H1 make antagonistic contributions to specific chromatin-dependent processes.

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Cystinosin/Ers1 functions in redox homeostasis in the early secretory pathway

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Cystinosis is an autosomal recessive inherited disorder caused by mutations in the *CTNS* gene, which encodes the highly conserved transmembrane protein cystinosin, a proton/cystine co-transporter at the lysosome membrane. However, reduction of cystine load in the lysosomes is insufficient to treat key disease symptoms, indicating that cystinosin performs additional disease-relevant functions. Here, we report that Ers1, the yeast homolog of cystinosin, localizes to and functions in the early secretory pathway. We provide evidence that Ers1 does not transport cystine. Ers1 genetically interacts with early secretory pathway recycling adaptors and redox-active Fe-S cluster-binding proteins. Notably, cystinosin-LKG, the extra-lysosomal localized splicing isoform of cystinosin, can functionally replace Ers1 in yeast. Collectively, our work uncovers a conserved role of cystinosin/Ers1 in the early secretory pathway, offering new molecular insights for understanding cystinosis pathology.

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Evolutionary rescue of human disease mutations

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Several thousand human disease-associated alleles have clear yeast orthologs, making yeast an ideal model system for advancing a basic understanding of human disease. We used CRISPR/Cas9 editing to generate 51 yeast strains with 11 human disease-associated variants, focusing on human disease genes involved in protein glycosylation, ribosome maturation, mitochondrial function, and RNA regulation. We find that most of these alleles recapitulate as fitness defects in yeast. To enrich for suppressors, we evolved these populations for 1,000 generations and performed whole genome sequencing. We identified both known and previously unknown suppressor mutations. For example, we identify mutations in *DPM1* and *RPS9B* that compensate for disease phenotypes of *ALG7* and *SDO1*, respectively. We are in the process of reconstructing strains harboring these evolved suppressor mutations and the original disease alleles in order to phenotypically assess their abilities to compensate for disease phenotypes. Our results will map local genetic interaction networks centered around disease-associated alleles, providing greater insights into disease phenotypes and informing potential targeted therapies for disease management in patients with rare disorders.

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The Single-Cell Spatiotemporal Proteome of Aging Reveals Structural Determinants of Age-Sensitive Proteome and Uncovers Molecular Interconnectivity among Hallmarks of Aging

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Aging is marked by a decline in cellular functions accompanied by widespread changes in mRNA and protein abundance, yet whether aging broadly remodels subcellular protein localization and concentration—and why some proteins change while others remain stable—remains unclear. This gap matters because cellular function depends not only on expression levels but also on correct spatial organization. Using yeast replicative aging as a model, we built a robotic pipeline to enrich old cells from 5,661 strains, acquired 90 million single-cell 3D images, and applied machine learning to map proteome-wide changes in localization, concentration, and aggregation throughout aging. This age-resolved single-cell atlas uncovers widespread proteome remodeling and rewiring of protein interaction networks, including previously unappreciated molecular changes that underlie major hallmarks of aging. Moreover, structural analysis reveals biophysical determinants of age-sensitive proteome remodeling across ages and species.

Together, these results reveal a structure-encoded intrinsic principle underlying spatial proteome breakdown during aging and integrating these spatial phenotypes reveals many molecular connections linking different hallmarks. Temporal analysis suggests that disorganization of nucleolar ribosome biogenesis, proteostasis decline, and mitochondrial dysfunction precede other hallmarks. Our findings substantially deepen the molecular underpinnings of aging hallmarks and provide a framework for linking them into a hierarchical sequence of cellular failures.

Keywords: aging, protein localization, protein concentration, protein structure, protein aggregation, hallmarks of aging, ribosome biogenesis, aging pathways.

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How do budding yeast die? A single-cell map of cellular dysfunctions

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Aging is the primary risk factor for a wide range of chronic and age-associated diseases. It manifests through a series of conserved molecular dysfunctions, often referred to as aging hallmarks. While these hallmarks have been extensively cataloged, how they interact and which relationships are causal remain poorly understood. In yeast, emerging evidence suggests that some cellular dysfunctions are connected through causal interactions that give rise to at least two distinct aging trajectories, centered on mitochondrial membrane potential loss and ribosomal DNA locus instability. However, it remains unclear how additional hallmarks integrate into these trajectories or whether they define alternative, competing pathways of cellular aging. To answer this question, we leverage recent single-cell RNA-sequencing datasets from aging Saccharomyces cerevisiae, and applied scRNA-seq factorization in order to identify gene expression programs and transcription factor (TF) responses associated with known and previously uncharacterized cellular dysfunctions. To further validate these predictions, we developed a transcription factor reporter toolkit covering more than 30 TFs predicted to be involved in aging. By longitudinally tracking TF reporters together with biosensors and organelle morphology, we systematically examined how dysfunctions emerge and interact across the full replicative lifespan in thousands of individual yeast cells across multiple environments. Our results indicate that aging-associated dysfunctions arise heterogeneously within isogenic populations and that environmental conditions influence which dysfunctions become dominant. In particular, we find that proteostatic loss, dysregulated nutrient sensing leading to apparent nutrient starvation, and activation of MAPK pathways such as the cell wall integrity and osmotic stress responses are common features of aging, yet arise heterogeneously across individual cells. These findings suggest that budding yeast cells experience many competing hazards that give rise to distinct paths to death. Together, this work outlines an approach for combining single-cell RNA-sequencing with longitudinal functional readouts to build an integrated map of cellular aging.

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Distinct amyloid structures formed for yeast prion [PSI+] while maturation in vivo

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Prions are self-replicating protein conformations that convert normal soluble proteins into fibrillar assemblies. One of the best-characterized prions, the yeast prion [PSI+], arises from a conformational conversion of the translation termination factor Sup35. Transient overexpression of the Sup35 in yeast strains possessing prion [PIN+] induces *de novo* formation of [PSI+], whereas treatment with guanidine hydrochloride (GdnHCl) to disable Hsp104 or overexpression of the chaperone Hsp104 eliminates it. Despite extensive study on [PSI+], the molecular basis underlying *de novo* formation and curing of [PSI$^+$] remains unknown. Here we established a method to isolate *de novo*-formed Sup35 fibrils from yeast and determined their polymorphism and structures at distinct stages using cryo-electron microscopy and atomic force microscopy. We found the intermediate ring fibrils differ from mature dot fibrils in conformation and composition. The ring fibril core (residues 60–131) adopts an M-shaped architecture, whereas the dot fibril core (residues 2–64 or 2–72) forms a Greek-key fold. Both fibril types are stabilized primarily by hydrophilic interactions among neutral polar residues, unlike pathological amyloids but consistent with functional amyloids, accounting for their structural reversibility. Our study provides a useful framework for investigating prion formation and propagation within living cells and suggests that intermediates may arise during the formation of pathological fibrils in human neurodegenerative diseases.

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Conserved arginine methyltransferase Hmt1 drives α-synuclein aggregate dissolution through a catalysis-independent pathway

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Abstract Protein homeostasis, the balance between protein folding, function, and clearance, is essential for cellular health, and its disruption is a hallmark of many neurodegenerative diseases, including Parkinson's disease. Arginine methylation, carried out by enzymes like Hmt1 in yeast and PRMT1 in humans, is best known for regulating transcription, translation, apoptosis and pre-mRNA splicing. In this study, we reveal a surprising, non-canonical role for these methyltransferases in controlling the aggregation and toxicity of α-synuclein, a protein lacking arginine and implicated in Parkinson's disease pathology. We find that under oxidative stress, Hmt1 and PRMT1 relocate from the nucleus into the cytoplasm. Using genetic, biochemical and imaging assays in yeast and mammalian cells, we observe that Hmt1/PRMT1 dissolve aggregates, promotes their removal through the ubiquitin–proteasome system, and reduces α-synuclein toxicity. Importantly, this activity does not require the classical methyltransferase function. Catalytically inactive mutants and a specific PRMT1 inhibitor both enhance aggregate clearance and improve cell survival. Together, our findings uncover an unexpected function for Hmt1 and PRMT1 in maintaining protein quality under stress, expanding our understanding of how cells cope with toxic protein assemblies and pointing to new directions for therapeutic strategies in protein aggregation disorders.

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Dissecting the functional consequences of missense mutations

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The number of identified genetic variants has increased exponentially over the last two decades, driven by advancements in high-throughput sequencing technology. Despite the improvements in variant identification, the functional importance of nearly all variants remains unclear. This is particularly true for missense variants, as their effect can range from neutral to a complete loss of gene function. In addition to the impact of a mutation on the affected protein itself, its deleteriousness is also determined by the genetic or environmental context in which it operates. For instance, high temperature or loss of a chaperone may increase the deleteriousness of destabilizing mutations. A complete understanding of the functional consequence of a missense variant will thus require untangling both its effect on the mutated protein, as well as the influence of the context.

To begin to distinguish these effects, we are systematically testing the fitness of millions of missense mutants across environmental and genetic contexts. We are using a CRISPR-Cas9 based method, known as MAGESTIC, to mutate all residues in 132 essential yeast genes into every other amino acid in a pooled format. The pool of edited cells is then cultured in different conditions, and the fitness consequences of each variant determined using sequencing to quantify the relative abundance of each mutant in the population over time. Initial results show that most nonsense mutations are depleted from our screen, whereas synonymous controls tend to be tolerated in all tested conditions, giving us confidence in the fitness phenotypes detected in our experiments. Furthermore, we are identifying conditional mutants that are tolerated under standard growth conditions but not in other contexts. For example, mutations on the interaction interface between the kinetochore protein Spc24 and its binding partner Spc25 are tolerated at 30°C but not at 37°C, indicating that these variants destabilize the interaction.

Overall, our large-scale analysis is identifying critical residues within 132 proteins, providing new insights into protein structure-function relationships, and identifying new conditional mutants that represent a powerful resource for the yeast community. Ultimately, the gathered knowledge will aid the interpretation of variants detected in genomes, including in genes associated with human disease.

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Genome-wide base editor screening identifies thousands of functional variants in yeast

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A central goal of genetics is to define the relationships between genotypes and phenotypes. However, genetic mapping approaches often lack the resolution to pinpoint causal variants, and deep mutational scanning is difficult to scale across an entire genome. Here, we present a deep genome-wide CRISPR base editor screen in *Saccharomyces cerevisiae*, deploying 356,323 guide RNAs to introduce single and multi-nucleotide variants across coding and regulatory regions of the genome. We identified 8,094 variants with significant impacts on fitness. The rate of significant fitness effects was highest for stop codons in essential genes, followed by stop codons in non-essential genes and non-synonymous variants in both classes of genes, with synonymous variants showing the lowest rate. These results are consistent with well-established patterns of variant impact across species. We identified 918 non-coding variants that influenced fitness, with gene-proximal variants showing larger effects than gene-distal variants, consistent with the expected role of proximal regulatory regions in controlling gene expression. Among 82,230 variants also observed in natural yeast populations, fitness effects were smaller than those of unobserved variants, and of the 1,280 natural variants with significant effects, common variants had smaller effects than rare ones, consistent with purifying selection. We identified 46 naturally occurring variants that significantly increase fitness, representing candidates for positive selection in yeast populations. At the gene level, our screen revealed modulators of base editor efficiency, offering insights into host factors that influence genome modification. Additionally, variants classified as pathogenic in humans at positions conserved between yeast and humans showed larger fitness effects than those classified as benign. This result highlights the potential of yeast variant screens to inform clinical variant interpretation. Together, these results establish a genome-wide map of variant function in a eukaryotic organism and demonstrate that large-scale base editor screens can connect sequence variation to phenotype at nucleotide resolution.

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Chemogenomic profiling of diverse *Saccharomyces cerevisiae* strains using BarMix: a novel CRISPR-Cas9 marker-less barcoded library

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Phenotypic diversity, expression, and penetrance vary according to both genetic and non-genetic factors, and gene-trait interactions drawn from one individual or strain may generalize poorly across a broader population. Drug sensitivity and efficacy, for example, is rarely uniform across individuals and often shows dependency on genetic background. While *Saccharomyces cerevisiae* is a powerful model organism for genotype-phenotype discovery, mutant strain libraries commonly comprise a limited genetic background, constraining their ability to model gene-trait diversity. Here, we introduce BarMix, a novel barcoded library of diverse *S. cerevisiae* strains constructed and validated for high-throughput chemical genetic screens. Using CRISPR-Cas9, distinct marker-less genetic barcodes were integrated into the genomes of 262 strains representing multiple phylogenetic clades of wild, domesticated, and admixed origins. To evaluate interactions between natural variation and chemical response, the pooled library was quantitatively profiled via barcode-sequencing against 33 small-molecule compounds with well-known and diverse bioactivity, including clinically approved antifungals and pharmaceuticals, agricultural fungicides, and chemical probes routinely used in biomedical research. Chemical fitness signatures strongly differed between domesticated and wild clades, particularly for stresses impacting ergosterol biosynthesis, DNA alkylation, microtubule depolymerization, TORC1 inactivation, and membrane permeability. We identified 281 chemogenomic interactions through genome-wide association analysis and validated 7 of these associated alleles through allele-swap experiments, including variants in genes that modulate multidrug export (*YRR1*), tRNA-specificity (*ArgRS*), small-molecule methylation (*CRG1*), and microtubule structure (*TUB2*). Several alleles further displayed incomplete penetrance across strains, indicating complex trait architecture. Finally, we demonstrate that the inclusion of admixed strains into genome-wide association design enhances variant detection in *S.*

cerevisiae. Together, these results establish BarMix as a powerful platform for identifying genotype-phenotype interactions across phylogenetic diversity with broad applications in functional genomics and biotechnology, while highlighting the value of incorporating natural variation into model systems biology.

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The rise and fall of brewing yeasts: Harnessing experimental evolution to select for increased flocculation

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Flocculation is the process by which microbial cells aggregate into dense clumps after initially growing as single, dispersed cells; the process is mediated by changes in the cell wall that occur in response to nutritional changes in the medium during growth. This trait is of major interest to the brewing industry because highly flocculent strains settle rapidly, reducing the need for filtration and shortening clarification times. Ideally, flocculation occurs only near the end of fermentation, allowing yeast cells to remain dispersed while efficiently utilizing fermentable sugars. However, many commercial brewing strains exhibit either low overall flocculation or premature flocculation during early or mid-fermentation stages.

Using a German-style lager strain and a West Coast-style ale strain, we performed experimental evolution studies designed to obtain yeast strains showing increased flocculation while still maintaining their original fermentation performance and sensory characteristics. For each strain, long-term evolution experiments were performed in triplicate using small-scale (3–5 mL) cultures grown in sterile 10% malt extract. After one day of growth for the ale strain or two days for the lager strain, cultures were briefly centrifuged at 100 × g, and a small volume from the bottom of the tube was transferred to fresh medium. This selective regime was repeated until pronounced increases in clumping behavior were observed, which occurred after more than 50 transfers for both strains.

From these experiments, we isolated novel clones from both the ale and lager backgrounds that display markedly increased flocculation. Whole-genome sequencing of evolved clones and their respective ancestor strains was performed, and we have identified nucleotide substitutions and copy number changes associated with the evolved phenotype. In the evolved lager strain, we identified a single nucleotide substitution in the *Lg-FLO1* gene that was absent from the ancestor. *Lg-FLO1* encodes a flocculation protein found in many ale and lager strains that confers a “NewFlo” phenotype, in which flocculation occurs only after most or all fermentable sugars have been depleted. The identified mutation results in a single amino acid substitution near a Lg-Flo1-specific loop in the sugar-binding region of the protein, suggesting a potential mechanistic basis for the enhanced flocculation phenotype.

Ongoing CRISPR-mediated genome editing experiments are aimed at introducing this mutation into the ancestral lager strain to directly test its causal role. If successful, this may indicate that we can quickly introduce strong “NewFlo” flocculation behavior into desired yeast strains. Together, these results demonstrate the utility of combining experimental evolution with targeted genetic modification to develop novel brewing yeast strains with improved industrially relevant traits.

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Generation of an isobutanol biosynthesis pathway library in *Saccharomyces cerevisiae* using LoxP–Cre recombination

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Isobutanol is a promising biofuel due to its higher energy density and compatibility with existing fuel infrastructure. Engineering industrial *Saccharomyces cerevisiae* is an attractive strategy because of its robustness and tolerance to toxic alcohols and acidic conditions, which makes it suitable for large-scale fermentation. However, in contrast to ethanol production, rational optimization of the heterologous isobutanol biosynthetic pathway has proven challenging. To address this, we use SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution) to generate diverse pathway variants. Using Cre–loxP recombination, SCRaMbLE generates structural variation by reorganizing transcriptional units, extending its application beyond genome restructuring in synthetic yeast to targeted pathway evolution.

We constructed a yeast shuttle vector (pIBA16) carrying five heterologous enzymes from the valine biosynthetic and Ehrlich pathways: acetolactate synthase (ALS), ketol-acid reductoisomerase (KARI), dihydroxy-acid dehydratase (DHAD), keto-acid decarboxylase (KIVD), and alcohol dehydrogenase (ADH). This pathway is designed to be growth-linked by coupling glycolytic pyruvate to isobutanol production in a pyruvate decarboxylase (PDC)-deficient host, thereby redirecting carbon flux away from ethanol formation.

Our host strain (GLBRCY1960), a xylose-consuming S. cerevisiae background, was further engineered by deleting *PDC1*, *PDC5*, *PDC6*, and *GPD2* to generate strain yHSCC140. In this strain, xylose consumption and growth are preserved, whereas glucose utilization is severely impaired. The absence of ethanol production and pyruvate accumulation suggests effective redirection of carbon flux, supporting its suitability as a chassis for isobutanol pathway selection.

Following transformation, pIBA16 was SCRaMbLEd in the engineered host, and variants were selected for growth on glucose. Because growth is coupled to isobutanol pathway function, this selection is expected to enrich for pathway configurations that enhance isobutanol production. By screening this diversified library, we aim to identify high-producing variants without relying solely on rational design. This work demonstrates the potential of SCRaMbLE as a generalizable platform for heterologous pathway evolution and metabolic engineering in *S. cerevisiae*.

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Synonymous recoding enables one-step whole chromosome assembly and substitution in yeast

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Efficient methods for assembling and replacing entire chromosomes are essential for synthetic genomics, yet synthetic *Saccharomyces cerevisiae* chromosomes have historically been built using random assembly adapters or iterative segment replacement strategies. Here, we describe an approach in which synonymously recoded genes serve as assembly adapters. Because the biological effects of synonymous recoding remain debated and the degree of recoding needed for assembly is unclear, we tested two strategies. Wobble-based recoding replaces each codon with a synonymous alternative recognized by the same tRNA through wobble base pairing, preserving tRNA usage across the gene. Congeneric synonymous recoding instead uses codon differences between orthologous proteins in *S. cerevisiae* and *S. eubayanus*, introducing *S. eubayanus* codons at synonymous positions while maintaining the *S. cerevisiae* amino acid sequence wherever the two species differ at the protein level. Both strategies alter more than one-third of codons. Applying these approaches to chromosome I, we assembled complete recoded chromosomes from more than 55 synthetic fragments in a single transformation. Congeneric recoding enabled both chromosome assembly and replacement of the native chromosome in the same step, recoding more than 65 genes, whereas wobble-based recoding supported assembly but did not exclude the native chromosome. These results establish synonymous recoding as a practical strategy for streamlined assembly of synthetic chromosomes and opens up new avenues for accelerated design-build-test cycles with non-natural chromosome designs.

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Synthetic Genome Expansion Reveals Physiological Consequences of Non-coding DNA

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Genome size varies dramatically across eukaryotes, largely due to differences in non-coding DNA content, yet the physiological consequences of excess non-coding DNA ('junk') remain poorly understood. To directly test how non-coding DNA abundance influences cellular physiology, we engineered a scalable system in *Saccharomyces cerevisiae* that enables controlled expansion of genome size while maintaining the endogenous genome constant. Using sequential yeast artificial chromosome (YAC)-chromosome fusions containing largely non-coding human DNA, we generated strains with up to ~12.7 Mb of additional DNA, effectively doubling the native yeast genome.

Genome expansion produced a proportional increase in cell size and a reduction in growth rate, primarily through delayed G1/S cell-cycle progression. Multi-omics measurements revealed pervasive transcription of the introduced non-coding DNA, while total cellular RNA polymerase II and protein concentrations remained approximately constant. Spike-in normalized RNA-seq and ChIP-seq showed that RNA polymerase II occupancy was redistributed from endogenous yeast genes to the added non-coding sequences, leading to a decrease in global mRNA concentration. Ribosome profiling and mass spectrometry indicated that these transcripts are largely non-productive, with minimal translation into detectable proteins.

To explain these observations, we developed a quantitative mathematical model in which non-coding DNA competes with endogenous genome for limiting transcriptional machinery. The model accurately predicts the redistribution of RNA polymerase II, reduced mRNA output, and the resulting decline in growth rate. Together, our results demonstrate that excess non-coding DNA acts as a sink for transcriptional resources, reshaping gene expression and cell physiology. This synthetic genome-expansion platform provides a quantitative framework for dissecting how genome composition constrains cellular function and offers mechanistic insight into the relationship between genome size, transcriptional capacity, and cell size across eukaryotes.

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Determining how the cell cycle regulator Bck2 controls cell size

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Cell size is a fundamental aspect of cellular physiology, and deviations from an optimal size range can lead to pathological phenotypes such as senescence and cell-cycle arrest. In the budding yeast *S. cerevisiae*, size control occurs primarily in G1, where the transition from G1 to S phase ("*Start*") is triggered by cell growth and size signals. The protein Bck2 has long been implicated in regulating the cell size threshold at *Start*, as cells lacking Bck2 become large. However, the mechanism by which Bck2 regulates cell size remains unclear.

Here, we report that Bck2 associates with promoters of genes regulated by SBF, the central transcription factor that activates *Start* and controls the expression of many cell cycle-dependent genes. These promoter interactions likely occur through SBF, as depletion of SBF from the nucleus results in the loss of Bck2 from SBF-regulated promoters. We identified the N-terminal region of Bck2 as necessary for this interaction. The association between SBF and Bck2 was further supported by single-molecule experiments measuring the diffusivity of Bck2 in the nucleus. SBF depletion increased the diffusion coefficient of Bck2, consistent with reduced chromatin association. Because this model predicts that Bck2 must function in the nucleus, we examined the requirement for Bck2 nuclear localization. Nuclear depletion of Bck2 phenocopied *bck2*Δ. Live-cell imaging revealed that Bck2 is enriched in the nucleus during G1 and exported following *Start*. Together, these results support a model in which Bck2 associates with DNA-bound SBF to regulate the *Start* transcriptional program and thereby control the cell size threshold for cell-cycle entry.

Previous work showed that SBF also binds the cell cycle inhibitor Whi5, whose concentration reflects cell size, and the cell cycle activator Cln3, whose concentration reflects cellular growth rate. Together with our findings, these observations support a multi-input model for *Start* regulation in which multiple pathways converge on the central hub SBF to determine whether cells commit to the division cycle.

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Control of cell growth and size by protein phosphatases

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Coordination between cell growth and division is essential for maintaining and appropriate cell size and preventing uncontrolled cell division. Without sufficient growth, proliferating cells arrest the cell cycle, which suggests that cells translate events of growth into biochemical signals that control the cell cycle. Aberrations in the signals that control cell growth and size are seen in nearly all cancers, yet the precise mechanisms cells use to measure growth, generate a signal proportional to growth, and relay that signal to cell cycle machinery remain obscure. Here, we aim to discover conserved mechanisms that link cell growth to the cell cycle machinery.

In budding yeast, the protein kinase Gin4 is required for normal control of bud growth and undergoes gradual multi-site hyperphosphorylation during growth that is dependent upon growth and proportional to the extent of growth. In conditions where the rate and extent of growth varies, the timing and extent of Gin4's phosphorylation and activity remain closely correlated with growth. In previous work, we identified protein kinases that are required for growth-dependent activation of Gin4. Here, we used Gin4 affinity chromatography to identify phosphatases that bind and regulate Gin4. This candidate approach led to the discovery of two conserved protein phosphatases that bind Gin4, oppose Gin4 phosphorylation, and are required for normal control of cell size. Together, the data suggest a model in which protein phosphatases oppose growth-dependent activation of Gin4, thereby setting a threshold that determines when sufficient growth has occurred for cell cycle progression.

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Adaptive control of TORC1 signaling during nitrogen limitation by Ait1 and the vacuolar transporter Vsb1

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The Target of Rapamycin Complex 1 (TORC1) is a central regulator of cell growth and metabolism, integrating signals from nutrients, energy status, and stress pathways. Although many upstream regulators of TORC1 have been identified, how these inputs cooperate to tune TORC1 activity across conditions remains unclear. Most studies have focused on binary transitions between nutrient-replete growth and acute starvation, leaving the mechanisms that adjust TORC1 activity during gradual changes in nutrient quality poorly understood.

To address this, we carried out a systematic analysis of TORC1 signaling in *Saccharomyces cerevisiae* during transitions from a high-quality nitrogen source to a poor nitrogen source and ultimately to complete nitrogen starvation. We found that cells moved to the poor quality nitrogen source proline permentantly enter a distinct partially inhibited TORC1 signaling state, we call the Low Nitrogen Adaptive (LoNA) state. Using phosphoproteomics, genetic perturbations, and quantitative signaling assays, we show that TORC1 activity is reduced but not eliminated in the LoNA state, leading to extensive reprogramming of nutrient transport, amino acid metabolism, and gene expression while still permitting growth. We also show that entry into this adaptive state is driven primarily by the regulators Ait1 and Gcn2. In contrast, complete nitrogen starvation engages additional regulators including SEAC/GATOR to fully inhibit TORC1, broadly dephosphorylate TORC1 targets, and drive entry into quiescence.

We further identify the vacuolar basic amino acid transporter Vsb1 as a new component of the TORC1 regulatory network. Vsb1 physically associates with TORC1 and is required for proper TORC1 repression during histidine and lysine starvation. Vsb1 also cooperates with Ait1 to allow cells to adapt to poor nitrogen conditions. Specifically, while wild-type cells establish the LoNA state following a shift from glutamine to proline, ait1Δ vsb1Δ double mutant cells instead exhibit a signaling response resembling complete nitrogen starvation. These results suggest that vacuolar amino acid storage and transport function as a buffering system that stabilizes cytosolic amino acid pools and helps cells distinguish nutrient limitation from starvation.

Together, these findings reveal a multilayered regulatory system in which signaling proteins and transporter-linked nutrient signals cooperate with vacuolar amino acid buffering to tune TORC1 activity and allow cells to distinguish nutrient limitation from true starvation. This work provides new insight into how eukaryotic cells dynamically balance growth and stress responses as nutrient availability deteriorates.

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Translational control of CAK and Cdk T-loop phosphorylation in response to growth in yeast

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Cyclin-dependent kinases (Cdks) require activating T-loop phosphorylation, a modification that is considered constitutive. Here, we examined the regulation of the Cdk-activating kinase, Cak1, in budding yeast. We measured Cak1 levels and the activating T169 phosphorylation of Cdc28 (the budding yeast Cdk) across various nutrient environments. We found that the abundance of Cak1 and the T169 phosphorylation is significantly reduced in cells that are proliferating very slowly or have entered quiescence. A small upstream open reading frame (uORF) in the CAK1 transcript represses Cak1 synthesis, especially in poor growth conditions. Eliminating the uORF increased Cak1 levels but did not alter proliferation kinetics under most laboratory contexts. Instead, it reduced the viability of quiescent cells and the fitness of slowly proliferating chemostat cultures. In cells lacking several type 2C protein phosphatases, which remove the T169 phosphorylation, there was a pronounced acceleration of initiation of cell division in the absence of the uORF in CAK1. Our results suggest an unexpected layer of control, impinging on the activating phosphorylation of the Cdk. The uORF-mediated repression of Cak1 synthesis directly couples protein synthesis to the activity of the core cell cycle machinery.

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The importance of regulated resource reallocation during dynamic environmental shifts in yeast

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All cells must be able to respond to stressful or unfavorable conditions. Many organisms activate generalized stress responses when met with such conditions, allowing them to maintain cellular homeostasis and protect against environmental stresses. Common to generalized stress responses is the induction of stress-defense proteins coupled to the reduction of growth-promoting proteins, including ribosomes. However, the distinct contributions of these coordinated processes remain challenging to untangle. To explore this, we used *Saccharomyces cerevisiae* (budding yeast) responding to salt as a model stressor. We employed molecular, genomic, and single-cell microfluidic methods to study the interplay between transient induction of stress-defense genes and repression of growth-promoting genes in the yeast environmental stress response (ESR). We found that loss of Msn2/4, stress-responsive transcription factors that induce stress-defense genes, led to increased growth during multiple different mild stress doses, however, at the expense of acquisition of tolerance to subsequent severe stresses. Conversely, loss of Dot6/Tod6, stress-responsive transcription factors that repress growth-promoting genes, delayed acclimation to stress, showing that gene repression accommodates the cost of the Msn2/4 response. Furthermore, we found that Msn2/4 bind the *DOT6* promoter, play roles in regulating Dot6 abundance and activation dynamics, and are needed for complete repression of Dot6 targets and additional growth-promoting genes. Together, these findings show that Msn2/4 participate in regulating resource reallocation needed to induce own response, highlighting a conserved strategy of stress responses seen across diverse organisms.

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Analysis of essential proteins in yeast filamentous growth identifies the WASP homolog Las17 as a regulator of the Cdc42-dependent fMAPK pathway

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The budding yeast *Saccharomyces cerevisiae* undergoes filamentous growth, where cells differentiate into elongated and interconnected cells capable of invading and expanding into new environments. Many proteins regulate this eukaryotic differentiation response; however, the diverse group of proteins essential for growth has yet to be systematically explored. To identify new roles for essential proteins in filamentous growth, a collection of 337 temperature-sensitive (ts) alleles in 320 essential genes was generated in a strain that undergoes filamentous/invasive growth. This resource was used to compare conditional essentiality among genetically distinct yeast strains and identify new roles for essential genes in the filamentation response. Functional tests showed that >40% of essential alleles displayed phenotypes in at least one test. To identify new regulators, key alleles were tested for effects on the Mitogen-Activated Protein Kinase (MAPK) pathway, a central regulator of filamentous growth. We found sixteen new regulators of the fMAPK pathway spanning six subcategories. One protein was the Wiskott-Aldrich Syndrome Protein (WASP) homolog Las17, which regulated the fMAPK pathway by coordinating the localization and levels of the sensor protein Sho1 and the Rho GTPase Cdc42. Las17 also functioned as a hub coordinating multiple aspects of filamentous growth. Collectively, the study reveals widespread roles for essential proteins in regulating eukaryotic cell differentiation. Due to their high conservation, essential proteins may play similar roles in regulating cell differentiation in other systems.

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Regulation of spindle disassembly in meiosis II

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S. cerevisiae diploids sporulate upon starvation, undergoing meiosis to create four haploid spores within the mother cell ascus. During meiosis II, the morphogenic processes required to create a spore begin with the initiation of the prospore membrane (PSM). The PSM grows to form the membranes that will ultimately encapsulate the four newly produced haploid nuclei. Closure of the PSM around the nucleus constitutes the cellularization event of meiosis, and this closure must be coordinated with events at the end meiosis II, including spindle disassembly. Interestingly, we found that the two pathways known to regulate PSM closure also regulate meiosis II spindle disassembly – the meiotic exit pathway (which involves the Hippo-like kinase Cdc15 and the STE20-family GCKIII kinase Sps1) and the APC/C protein degradation pathway (which involves the meiotic specific regulator, Ama1). At a mechanistic level, we find that the *CDC15-SPS1* pathway is required for the removal of Bim1 (an EB1 end binding protein also present on the mitotic spindle) from the meiotic spindle during meiosis II spindle disassembly, while *AMA1* is needed for the removal of Cin8 (kinesin-5) and Ase1 (a microtubule bundling protein also found at the mitotic midzone). In addition, we found that even when both these pathways were inactivated, some spindle disassembly still took place, suggesting additional pathways contribute. As *KIP3* (a kinesin-8 family member) has a key role in spindle disassembly during mitosis, we examined its contribution to this process during meiosis. We found that *kip3*Δ *sps1*Δ *ama1*Δ cells fail to disassemble their spindles. Thus, we find that spindle disassembly during meiosis II involves many of the same microtubule-associated proteins important during mitosis. However, the function of these proteins is subject to the control of at least two meiosis-specific signaling pathways.

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Fitness Landscapes Reveal Modular Phenotypes in Yeast Drug Resistance Evolution

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Understanding how mutations generate phenotypic diversity that shapes evolutionary trajectories remains a central challenge in evolutionary biology. The genetic tractability and experimental power of *Saccharomyces cerevisiae* make yeast an unparalleled system for mapping genotype-to-fitness relationships at scale. Here, we leveraged massively parallel experimental evolution to dissect the structure of drug resistance adaptation in yeast.

We tracked approximately 300,000 barcoded yeast lineages adapting to growth-inhibiting concentrations and combinations of fluconazole and radicicol. From these populations, we isolated ~1,000 adaptive mutants and quantified their fitness across 12 environments. Despite extensive genetic heterogeneity, genotype-by-environment (G×E) fitness responses collapsed into just six characteristic trade-off patterns. These results suggest that while the genetic basis of resistance is diverse, the accessible phenotypic space is constrained and modular.

To test whether fitness-defined clusters reflect underlying biological states, we performed single-cell RNA sequencing on mutants spanning distinct G×E classes. Mutants from different fitness clusters exhibited strikingly distinct transcriptional programs involving largely non-overlapping gene sets and divergent drug-response signatures. Moreover, mutants with environment-specific fitness deficits showed corresponding stress-response transcriptional states. Thus, clustering based solely on fitness measurements recovers underlying molecular phenotypes at single-cell resolution.

Together, these results demonstrate that yeast fitness landscapes encode information about hidden cellular states and that phenotypic space is structured into discrete, biologically meaningful modules. By combining the experimental power of yeast genetics, large-scale lineage tracking, and single-cell transcriptomics, this work provides a framework for interpreting evolutionary outcomes and suggests that adaptive trajectories may be more structured—and therefore more predictable—than previously appreciated.

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The Hidden Life of Yeast Viruses

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In recent years, sequencing studies have revealed a vast array of RNA viruses that infect many fungal species. These mycoviruses are well known to persist as endemic infections transmitted through cell division or fusion with no known extracellular route. Although often believed to be asymptomatic, mycoviruses are now understood to profoundly influence fungal phenotypes in highly consequential but poorly understood ways. Prominent examples include mycoviruses that induce hypo- or hyper-virulence in fungal pathogens that infect plants, insects, and humans. Thus, mycoviruses are better understood as spanning the symbiotic spectrum, contributing to extra-chromosomal 'missing heritability' that shapes fungal phenotypes. Here we investigate mycovirus controlled phenotypes at unprecedented scale using the budding yeast *Saccharomyces cerevisiae*. We utilize a collection of 1,011 yeast isolates from across the planet, each with a sequenced genome, transcriptome, and comprehensive phenotypic profile. Through computational analysis of their transcriptomes, we establish the yeast RNA virome across the entire collection, including 14 new mycoviruses in yeast. Leveraging this new resource, we discover a robust virome-phenome association, including a particular role of mycovirus infection for progression through the sexual cycle (sporulation) as well as new viral encoded toxins and a novel toxin defense system. These findings reveal the tremendous potential of yeast for investigating mycovirus phenotypes, opening this burgeoning field to the unique strengths of this model organism.

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Synthetic Interspecies Hybrid Chromosomes Reveal Unexpected Phenotypes Due to Higher-Order Epistasis

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Even in budding yeast, one of the most powerful model organisms, identifying the genetic basis of trait differences between species remains a major challenge. Here, we present an updated version of CReATiNG, a method for assembling synthetic yeast chromosomes from natural DNA, that can be used to address a variety of biological questions, including the genetics of differences between yeast species. CReATiNG enables the synthesis of haploid cells containing hybrid chromosomes, such as chromosomes composed of a mix of segments from S. cerevisiae and S. paradoxus. By combining hierarchical in vitro and in vivo assembly steps, we increase the number of recombination breakpoints on these synthetic hybrid chromosomes by 400%. By generating and phenotyping 1,024 strains containing hybrid S. cerevisiae-S. paradoxus Chromosome I variants, we show that this expanded recombination gives rise to novel multigenic traits that are not observed in either parent species and likely involve genetic interactions among five or more loci. We are now working to identify the specific causal genes underlying these unexpected traits. These results illustrate how synthetic chromosomes can facilitate evolutionary genetic research, enabling the discovery of new complex traits and their high-resolution genetic dissection.

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Epistasis between gene expression noise and functional mutations shapes cellular fitness

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Epistasis is the phenomenon in which the phenotypic effect of a mutation depends on the genetic background. Beyond interactions among coding mutations, noncoding mutations—such as those affecting promoter activity—can also contribute to epistatic effects. Notably, the phenotypic consequence of a coding mutation can be deleterious, neutral, or beneficial depending on its expression level, indicating epistasis between regulatory and coding changes. Even within genetically identical populations, the abundance of any given protein varies among individual cells, a phenomenon known as gene expression noise. Such variability can enable rapid responses to fluctuating environments and facilitate phenotypic evolution. We therefore hypothesize that epistasis between expression noise levels and functional mutations can arise within clonal populations. To test this idea, we performed a fitness screen of mutant clones generated by random mutagenesis in budding yeast using a genetic background that allows tunable control of noise levels. Under high-noise conditions, the mutant library exhibited increased phenotypic diversity, suggesting that expression noise could enhance phenotypic heterogeneity. We then identified mutant clones whose fitness differed across noise levels. Intriguingly, one clone displayed a fitness defect that was strongly potentiated by increased noise. Using quantitative trait locus sequencing (QTL-seq), we pinpointed a mutation in *RNT1*, the gene encoding the sole RNase III in budding yeast, as the causal variant. Taken together, our findings reveal epistasis between gene expression noise and functional mutations and suggest that expression noise can uncover cryptic fitness effects, thereby contributing to phenotypic diversity during evolution.

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Gene duplications, molecular degeneracy, and diversification in the evolution of an essential metabolic step

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Every gene and genetic pathway evolves. Yet, despite this evolution, many essential phenotypes remain conserved across vast evolutionary scales. The conservation of phenotypes often disguises substantial evolutionary variation in the underlying genetic architecture and offers a powerful way to study important questions: why do genetic architectures for equivalent functions vary in their complexity, and what molecular mechanisms enable and constrain the evolution of this variation? Here, we addressed these questions by investigating the evolutionary elaboration in the genetic architecture of an essential metabolic step in glycolysis, the interconversion of glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate. In *Saccharomyces* yeast, this step can be catalyzed by three *GAPDH*-encoding genes that arose through gene duplication events over 100 million years ago. We found that all paralogs have retained their ancestral catalytic capacities but have evolved to have different effects on growth through *cis*-regulatory changes. We mapped the cis-regulatory differences that largely explain expression variation among the paralogs and found that they altered the recruitment of individual and cooperatively activating transcription factors; further, our findings suggest that these changes likely happened in an ordered way after each gene duplication, making it possible for crucial regulatory connections to be conserved overall even as the specific connections became shuffled among the different copies. In support of such a scenario, we found that the cells' growth and homeostatic state are sensitive only to overall *GAPDH* activity and indifferent to the genetic mechanisms through which that activity is altered. Lastly, we find that ongoing coevolution between *GAPDH*s continues to alter the genetic architecture of this conserved metabolic step even among closely related populations and species of *Saccharomyces* yeast. Collectively, our results show that simple principles—the degeneracy of molecular mechanisms for specifying a function, and the indifference of selection to which mechanisms are used—can account for the evolution of varying levels of complexity in genotype-phenotype relationships, even for the most essential functions.

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The link between virulence, amoeba predation, and multicellularity in yeast

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Many disease-causing microbes are not obligate pathogens; rather, they are environmental microbes taking advantage of an ecological opportunity. Selection in the open environment may favor traits that incidentally lead to pathogenicity, or serve as pre-adaptations for survival in a host. An example is microbial adherence, which can increase fitness in harsh environments, but is also associated with pathogenicity. A major selective force that has been proposed to favor dual-use traits is amoeba predation, which may inadvertently favor resistance to phagocytes in the immune system. Here, we leverage the power of the 1011 *Saccharomyces* genomes collection to determine whether multicellular adherence phenotypes and amoeba predation are associated with virulence across a broad sampling of yeast ecology and geography. We assayed the entire panel for multicellular phenotypes and for predation avoidance using the amoeba *Dictyostellium discoideum*; virulence was estimated using larvae of the wax moth, *Galleria mellonella*. We found significant variation in multicellularity and predation avoidance, but no association between them. Multicellularity was more prevalent in human-derived isolates and isolates that expressed hyper-multicellularity had increased virulence compared to those that exhibited no multicellular phenotypes. In contrast, isolates with the lowest predation rates were more likely to come from natural environments, and rates of predation avoidance were not associated with virulence. Our results highlight the complex factors that may be shaping virulence in opportunistic fungi and demonstrate the power of using biomedical models for studies in evolutionary medicine.

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Exploring Coronavirus Host Range by Deep Homolog Scanning of ACE2

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SARS-CoV-2 infection is initiated through interaction between the viral spike protein and its cellular receptor, angiotensin-converting enzyme 2 (ACE2). Because ACE2 is conserved and expressed across diverse vertebrate species, spike–ACE2 compatibility is a key determinant of host range and a useful proxy for identifying potential zoonotic reservoirs. Although bats are considered natural reservoirs of SARS-related coronaviruses, the identities of intermediate and alternative hosts remain poorly defined, and direct infection or field studies across hundreds of species are not feasible at scale.To address this limitation, we developed a high-throughput experimental platform that integrates large-scale oligonucleotide synthesis with yeast surface display to perform a deep homolog scan of ACE2 across the vertebrate tree of life. Using this approach, we constructed a library of chimeric ACE2 receptors representing more than 800 vertebrate species and systematically quantified their functional compatibility with SARS-CoV-2 spike proteins. This screen identified both permissive and non-permissive species and revealed unexpected candidates including elephant shrew and meerkat that supported spike binding and viral entry despite being predicted as low risk by computational models. We further applied this platform to assess how SARS-CoV-2 evolution has reshaped host range by comparing ACE2 binding profiles across viral variants. We observed clear, variant-dependent gains and losses of compatibility among rodents, primates, and artiodactyls, indicating that viral evolution has altered host range in a lineage-specific manner. In parallel, we are leveraging these data to define the molecular determinants underlying species-specific susceptibility at the spike ACE2 interface.Together, this work establishes a scalable framework for systematically mapping coronavirus host range, refining zoonotic risk predictions, and monitoring how viral evolution may shift potential reservoirs over time.

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The RCDosome: How Cyclin C Drives Regulated Cell Death in Yeast

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Oxidative stress triggers regulated cell death (RCD) in *Saccharomyces cerevisiae* through a mitochondrial outer membrane permeability (MOMP)-associated mechanisms coordinated by cyclin C (Cnc1). Under non-stress conditions, Cnc1 interacts with the Mediator Kinase Module (MKM) to repress stress responsive genes transcription. However, oxidative stress induces Cnc1 translocation to the cytoplasm where it interacts with the dynamin-related

GTPase Dnm1 to drive mitochondrial fission. However, we previously found that although Dnm1-Cnc1 interaction is required for RCD, mitochondrial fission itself is not. In the present study, we identified the small G-protein Rho5 as a critical effector linking Cnc1-Dnm1 signaling to mitochondrial permeabilization. Genetic epistasis and co-immunoprecipitation analyses showed that Rho5 and the ER-mitochondria encounter structure (ERMES) complex act downstream of Cnc1 to enable productive Cnc1-Dnm1 complex formation. Furthermore, activated Rho5 ($Rho5^{G12V}$) bypassed the requirement for Cnc1 or Dnm1, placing Rho5 downstream of these proteins in the death cascade. Conversely, ERMES integrity remained essential for Rho5-driven cell death, indicating that ER-mitochondria contact sites serve as execution platforms. Rho5 co-immunoprecipitated with the mitochondrial porin Por1 in a Cnc1- and ERMES-dependent manner. This association correlated with Por1 degradation, MOMP and mitochondrial Ca^{2+} influx. We further demonstrated that vacuolar membrane permeabilization (VMP) preceded and was required for MOMP positioning the vacuole upstream of mitochondrial collapse. This model was supported by temporal and genetic analyses revealing that VMP rarely occurred without subsequent MOMP, whereas MOMP seldom occurred in the absence of VMP. These findings suggest vacuolar permeabilization is a prerequisite for MOMP and subsequent RCD. Together, our findings define a stress-induced Cnc1-Dnm1-Rho5 signaling module that coordinates MOMP at ER-mitochondria contact sites with vacuolar destabilization to drive regulated cell death in yeast.

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Human mixed myopathy causing point mutations completely block Hsp104-mediated yeast prion elimination when made in the yeast J-domain protein Sis1 Justin Hines, Lauren

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Yeast cell populations can be efficiently cured of the prion [*PSI*$^+$] by a controversial mechanism that requires at least two J-domain proteins (JDPs) called Sis1 and Apj1. However, the requirement for JDPs is specific to strong, but not weak, variants of [*PSI*$^+$], implying distinct mechanisms for curing specific prion variants. Strong variant curing requires the GF-rich region of Sis1 whereas the GF-rich region of Apj1 is dispensable. Presumably the J-domain of Sis1 is also required but no direct data currently supports that assertion. Several mutations in these same regions (J-domain and GF-rich) of the human Sis1 homolog DNAJB6 cause mixed myopathies including Limb Girdle Muscular Dystrophy Type D1. We assessed whether seven myopathy-causing point mutations could disrupt curing when made in Sis1. While none impaired the propagation of strong [*PSI*$^+$] variants, three spatially proximal residues dramatically impaired Hsp104 curing. No point mutations in Sis1 have previously been reported that disrupt Hsp104-mediated prion elimination. Because two mutations that block curing are in the J-domain, these results demonstrate for the first time that Sis1's J-domain is necessary for Hsp104 curing and infer that Sis1 acts with an Hsp70 partner in this mechanism. Additionally, the finding that just a single residue change in the GF region of Sis1 can disrupt curing while this same region of Apj1 is dispensable underscores that Apj1 and Sis1 act through different parts of the respective proteins in this process. All mutations also propagated weak [*PSI*$^+$] variants but no mutation impacted their curing, further supporting the hypothesis that weak [*PSI*$^+$] variants are cured by a biochemically distinct mechanism. Finally, because the prion [*RNQ*$^+$] is more sensitive to alterations in Sis1's GF region than [*PSI*$^+$], we asked whether these mutations might affect its propagation. No effect on [*RNQ*$^+$] was observed. In summation, spatially proximal mutations in human DNAJB6 that result in mixed myopathies alter JDP function in a way that specifically affects Hsp104 prion curing in yeast. Understanding this JDP function may help to elucidate the mechanism by which alterations of DNAJB6 result in disease.

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A transiently heritable functional prion is formed under microtubule stress conditions in *Saccharomyces cerevisiae*. Irina L Derkatch, Susan W Liebman, Olivia Hall, Irina Alexandrova, Melanie Ha

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Earlier studies identified a microtubule-associated complex that encompasses a two-protein amyloid co-aggregate ([*PUB1 / SUP35*]), components of protein synthesis machinery, and *TUB1* mRNA. We hypothesize that this is a functional prion localizing tubulin synthesis to microtubules. To test this hypothesis, we asked if microtubular stress leads to prion formation. Indeed, stress caused by microtubule depolymerizing fungicide Benomyl leads to the establishment of Benomyl resistant state (Ben^R) in ~10% cells. As expected of a prion, this state is heritable during growth in the absence of the drug. As expected of a functional prion, this heritability is transient: the Ben^R state is gradually lost after ~40 generations of growth in the absence of the drug. To test if the Ben^R prion is equivalent to [*PUB1 / SUP35*] amyloid, we asked if Ben^R formation depletes Sup35 protein from other cellular locations. One such location is cytoplasmic ribosomes, because Sup35 is a translation termination factor. Indeed, establishment of the Ben^R state led to nonsense suppression, a readthrough of a premature stop codon in the *ADE1* gene. The other location for Sup35 is the selfish [*PSI*$^+$] prion occasionally found in laboratory and wild yeast. As predicted for the competition of Ben^R for Sup35, we found that growth on Benomyl cures weak and inhibits strong [*PSI*$^+$] variants, and that the presence of strong [*PSI*$^+$] variants inhibits the induction of Ben^R. To identify other components of the [*PUB1 / SUP35*] complex, we obtained a mutant that makes yeast sensitive to Benomyl. This sensitivity is likely through inhibiting the formation of the complex because the mutation also affects aggregation of Sup35 into [*PSI*$^+$] and affects stability of centromeric plasmids, which depends on microtubular cytoskeleton. Based on the comparison of whole genome sequences of the mutant and wild type strains, we conclude that the mutation is in the *BUL1* gene thus implicating the alpha-arrestin ubiquitin ligase adaptor into the control of microtubule cytoskeleton. Based on other findings, we predict that an orthologous complex is formed in mammalian neurons where it contributes to fear memory.

72A

Investigating spatial dynamics of sHSPs in chronologically aging yeast Daniel A Escobar-Osorio[1,2], Long Duy Duong[3], Kevin A Morano[3]

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A hallmark of aging is the decrease in protein homeostasis (proteostasis) which can lead to accumulation of misfolded and damaged proteins that contribute to age-related diseases. As part of the first line of defense, small heat shock proteins (sHSPs) hold a crucial role in mitigating aggregation by acting as chaperone holdases, sequestrases, and aggregases. They interact with misfolded or damaged proteins, direct them to specific cellular locations, and form larger substrate-chaperone complexes presumably to minimize deleterious effects of uncontrolled protein aggregation. However, the precise roles of

sHSPs in maintaining proteostasis during aging remain incompletely understood. In this study, we investigated the dynamics of the three *S. cerevisiae* sHSPs, Hsp26, Hsp42, and Btn2, all of which share the conserved a-crystallin domain, in chronologically aging/stationary yeast cells using previously generated GFP fusions. In line with earlier studies, we confirmed that both Hsp42 and Hsp26 form protein foci in stationary-phase cells. In contrast, we found that Btn2 expression is very low and that Btn2 does not accumulate in large foci. Interestingly, Hsp42 and Hsp26 foci show partial colocalization, and a larger fraction of Hsp26 foci overlap with Hsp42 than vice versa. Deletion of *HSP42* reduces both the proportion of cells containing Hsp26 foci and the size of those foci, whereas the absence of *HSP26* does not affect Hsp42 foci formation, suggesting that Hsp26 foci formation partly depends on Hsp42 in chronologically aging yeast. We also found that the Prion-Like Domain (PrLD) at the N-terminus of Hsp42 is essential for foci formation in stationary phase. Notably, replacing the N-terminal domain (NTD) of Btn2 with the Hsp42 PrLD enables Btn2 to form foci. However, the Hsp42 PrLD alone is unable to direct GFP into foci and requires the a-crystallin domain to do so. Similarly, the NTD of Hsp26 is critical for Hsp26 foci formation; however, in contrast to the Hsp42 PrLD, the Hsp26 NTD can form foci when fused to GFP in the absence of the a-crystallin domain, suggesting distinct interactions mediate foci formation of small heat shock proteins. Taken together, our findings indicate that both Hsp26 and Hsp42, by virtue of their N-terminal domains, likely play a major role in the sequestration of proteins in chronologically aging yeast cells. Identifying the protein complement of these different structures will be key to understanding how protein sequestration occurs in aging yeast cells, as well as potential impacts on cell fitness and adaptation to environmental stress.

73A

Amino Acid-Dependent Modulation of Replicative Lifespan and Distinct Aging Trajectories in Saccharomyces cerevisiae

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Studying the replicative lifespan (RLS) of S. cerevisiae has been instrumental in providing many fundamental and evolutionarily conserved insights about cellular aging mechanisms in eukaryotes (Shabestary et al., 2025). Building upon this model, our research focuses on dissecting the intricate interplay between genetics and environmental factors, specifically nutrient availability, in determining RLS.

To systematically investigate the effect of environment, we leveraged our high-throughput technologies: the Yeast Lifespan Machine (Thayer et al., 2022) for automated, long-term single-cell RLS analysis and the Ministat Aging Device (Hendrickson et al., 2018) for purifying large populations of aged mother cells in a controlled environment. Using these tools, we discovered that RLS can exhibit a dramatic variation, spanning a greater than four-fold range, depending on which single amino acid is supplied in the growth medium.

We characterized the molecular signaling pathways responsible for mediating these differential RLS responses. Our data indicate that the SPS (Ssy1-Ptr3-Ssy5), TOR (Target of Rapamycin), and GCN2/GCN4 (General Control Nonderepressible) signaling cascades are all critically involved in sensing the specific amino acid environment and transducing these signals to modulate RLS. However, the relative contribution and regulatory role of each of these pathways vary depending on the particular amino acid present. In addition to these known nutrient-sensing pathways, we have identified a previously unrecognized role for lysine in regulating vacuolar function, which contributes to the modulation of RLS in its presence.

Importantly, we also identified distinct, competing aging trajectories within a population of genetically identical cells grown in a common environment. These trajectories represent different modes of physiological decline, acting as competing hazards that collectively determine the ultimate lifespan of individual cells (Hotz et al., 2026 and poster Shabestary et al.). This framework acknowledges the heterogeneity inherent in cellular senescence.

The presence, penetrance, and rate of progression of these distinct aging trajectories are differentially influenced by each of the single amino acids tested. This suggests that specific nutrient cues not only modulate the overall pace of aging but also bias the population toward specific modes of terminal decline. To elucidate the underpinnings of these trajectory-specific aging processes, we conducted systematic genome-wide screens of RLS in the presence of various amino acids. This comprehensive approach has revealed both common and distinct pathways that govern lifespan across multiple amino acid environments and within the context of the different, competing aging trajectories.

74A

Modulation of purine metabolism by solute carrier Tpo1 alters anticancer ruthenium complex resistance in yeast

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The clinically promising anticancer ruthenium complex indazolium *trans*-[tetrachlorobis(1*H*-indazole) ruthenate(III)], also known as KP1019, has been shown to cause genotoxicity, proteotoxicity, oxidative stress, and G2/M arrest in both *S. cerevisiae and* cancer cells. These findings suggest that budding yeast can be an effective model organism for further elucidating the cellular response to KP1019. A previous transcriptomic study indicated that KP1019 treatment increases expression of the promiscuous *S. cerevisiae* solute carrier Tpo1 (human ortholog: MFSD10). Here we use a GFP reporter to verify that KP1019 elevates *TPO1* expression and to demonstrate that this induction depends largely on transcription factor Pdr1. Paradoxically, given its role in drug efflux, loss of Tpo1 increases KP1019 resistance. To better understand the origin of this resistance phenotype, wildtype and *tpo1* null yeast were treated with and without KP1019 then subjected to RNA-seq analysis. In both wild-type and *tpo1* null yeast, KP1019 induced ribonucleotide reductase genes as well as genes involved in double-strand break repair. These findings support the conclusion that DNA damage is an important modality for KP1019's effects on cell growth and survival in both strains. Except for the loss of *TPO1*, no significant differences in gene expression were observed when comparing untreated wild-type and tpo1 null strains. However, in KP1019-treated samples, yeast lacking *tpo1* had ≥ 2-fold higher expression of many de novo purine synthesis genes relative to the wild-type control. Consistent with purine homeostasis being a modulator of KP1019 tolerance, we observed that yeast lacking the adenine deaminase Aah1 are resistant to KP1019. Future studies will examine the mechanism(s) by which purine homeostasis impacts KP1019 bioactivity as well as Tpo1's role in regulating stress-dependent changes in gene expression.

75A

Beyond glycolysis: 2-deoxyglucose depletes amino acids through α-arrestin-dependent transporter endocytosis

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Cancer cells depend on rapid nutrient uptake through plasma membrane (PM) transporters. Many undergo the Warburg effect—shifting from aerobic respiration to aerobic glycolysis—making them heavily reliant on glucose due to reduced ATP generation per glucose molecule. This glycolytic dependence creates a therapeutic window, where cancer cells are metabolically distinct from healthy cells.

2-Deoxyglucose (2DG), a toxic glucose analog that inhibits glycolysis, exploits this therapeutic vulnerability in cancer cells. 2DG has orphan drug status for treating glioblastomas, aggressive brain cancers with a median survival of 14.6 months post diagnosis. Despite clinical use, the full scope of 2DG's cytotoxicity remains unclear. Earlier studies from our lab discovered that 2DG triggers rapid endocytosis of glucose transporters, starving cells of glucose. α-Arrestins, a dynamic and understudied class of trafficking adaptors, regulate this 2DG-induced endocytosis of glucose transporters.

How widespread is 2DG-induced, a-arrestin-mediated endocytosis? We conducted a high-content imaging screen of all yeast PM proteins to identify which respond to 2DG and α-arrestins. Remarkably, nearly all amino acid transporters are rapidly removed from the PM and exhibit increased vacuolar trafficking upon 2DG treatment, consistent with elevated endocytosis. This suggested that 2DG-treated cells might also be starved for amino acids— another nutrient critical for rapidly proliferating cancer cells. Indeed, our metabolomics analysis demonstrate that nearly all amino acids are significantly decreased after 2DG treatment. These findings provide a mechanistic explanation for similar metabolomics observation, which found that amino acids were reduced in 2DG-treated human cancer cell lines. Finally, our data indicate that this 2DG-induced endocytic remodeling depends largely on α-arrestins, demonstrating that a network of α-arrestins become activated in response to this drug.

Our studies reveal widespread, α-arrestin-dependent membrane proteome remodeling in response to 2DG, removing not only glucose transporters but also amino acid transporters. We propose that this coordinated removal of nutrient transporters starves treated cells of both glucose and amino acids—metabolites critical for rapid cancer cell proliferation. These findings reframe 2DG's mechanism of action and open new avenues for understanding how targeting membrane trafficking could enhance its therapeutic efficacy.

76B

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 genetic 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 the 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 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 February 2026, 811 MO researchers have registered 17,778 genes of interest. With the aid of the computational inference built into the Registry, this translates to the coverage of 10,124 human genes. RDMM uses a committee process to identify and review potential clinician-MO researcher matches and approve $30,000 CAD in catalyst funding. Since 2014, we have made 132 clinician-MO scientist connections and funded 152 functional characterization proposals. We have also collaborated with several disease foundations to make targeted calls and co-funded an additional 47 clinician-MO researcher awards. 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.

In 2019, we established international linkages with emerging similar networks in Europe, Australia, and Japan. To facilitate community uptake, we made the RDMM Registry portable, customizable and linkable with other instances, and our committee structures and process freely available. We are willing to assist additional RDMM regional networks as needed, and to further support global collaborations. (https://rdmminternational.org/)

In our third round of funding (2022-2027), RDMM expanded the scope of model systems to include the use of human cell models, including primary patient-derived cells, cell lines derived from iPSCs, and organoids. The Canadian RDMM Network will continue to create meaningful collaborations between clinicians and MO researchers and advance RD research locally and globally.

77B

CTG clade-specific proteins of the *Candida albicans* RSC chromatin-remodeling complex possess the potential as novel physiological targets

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Candida albicans, a World Health Organization-categorized critical priority fungal pathogen, can opportunistically cause mucosal or systemic infections with a high mortality rate in the latter case. The emergence of resistance against existing anti-*Candida* drugs is a growing challenge, which therefore argues for further research to discover novel drug targets. The fungus uses morphological transitions and genomic plasticity to express its pathogenic traits. As the regulation of these mechanisms is predominantly based on differential transcription, robust chromatin remodeling is crucial to modulate dynamic DNA accessibility required for transcription. Mass spectrometry-based identification of the *C. albicans* RSC (Remodels the Structure of Chromatin) chromatin-remodeling complex by our group revealed the presence of two novel fungal CTG clade-specific subunits, Nri1 and Nri2. Due to their fungal-specific nature, it is intriguing to understand their roles in *Candida* biology. In this context, using various genetic, cell, and molecular biology techniques, we demonstrated the role of Nri proteins in regulating multiple cellular processes such as cytokinesis, spindle morphology, cell cycle progression, and mitochondrial function. RNA-seq analysis revealed that Nri proteins regulate the expression of genes controlling a broad range of cellular processes. Deletion mutants of these genes also exhibit altered pathogenic attributes such as defective morphological transition, adhesion, invasion, cell wall composition, and antifungal drug susceptibility. *nri* mutants are also completely avirulent in the mouse model for systemic candidiasis. Altogether, our results indicate that Nri proteins extensively influence the fitness and virulence of *C. albicans* and, thus, have the potential to be explored as antifungal therapeutic targets.

78B

A Yeast Model for the Functional Analysis of SRP54 Mutations Associated with Severe Congenital Neutropenia

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Mutations in *SRP54*, an essential subunit of the Signal Recognition particle (SRP), are associated with severe congenital neutropenia (Carapito *et al.* 2017, Bellanné-Chantelot *et al.* 2018, Juaire *et al.* 2021). SRP54 is a key subunit of the 6-protein SRP complex and is mainly associated with binding to the nascent signal peptide during cotranslational targeting to the endoplasmic reticulum (Berndt *et al.* 2009). These disease-associated mutations in SRP54 have been shown to disrupt GTP binding, leading to dominant-negative disease phenotypes (Bellanné-Chantelot *et al.* 2018, Juaire *et al.* 2021, Losievski *et al.* 2025). In this investigation, we characterize, using growth-based promoter shut-off yeast models, the impact of these disease-associated mutations have on *S. cerevisiae* growth. In doing so we find that the most common clinical mutations (T117del, T115A, and G226E) induce dominant-negative phenotypes when transposed to the yeast SRP54 structure (Long *et al.* 2024). Additionally, to establish a structural foundation for our model, we report the first X-ray crystal structure of yeast SRP54; by aligning this with the human ortholog, we provide a basis for interpreting the effects of known human mutations on yeast phenotypes. The ability of these models to replicate loss-of-function and dominant-negative phenotypes associated with disease-relevant *SRP54* mutations demonstrates their utility as a yeast disease model. Furthermore, this methodology may be useful for creating models to characterize disease-associated mutations in other components of the signal recognition particle.

79B

Mitochondria-lysosome coupling contributes to lysosome acidification and aging

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Nearly all cellular processes are pH dependent. The acidic pH inside the lysosome (vacuole in yeast) is essential for cellular content degradation, signaling, and autophagy. Defect in lysosome/vacuole acidification is a conserved hallmark of aging and age-related diseases. Traditionally, lysosome/vacuole is thought to import free protons (H^+) from the surrounding neutral cytosol. In this study, we uncovered a previously unrecognized, conserved lysosome/vacuole acidification mechanism, involving lysosomal/vacuolar uptake of H+ pumped out by mitochondrial electron transport chain through membrane contacts between mitochondria and lysosomes/vacuoles. Aging/senescence-associated disruption of mitochondria-lysosome/vacuole contacts causes lysosomal/vacuolar de-acidification, which can be reversed by expressing a linker to connect these organelles and through an asymmetry-dependent rejuvenation process in daughter cells. Preserving lysosomal acidification in senescent human cells prevents the induction of major senescence-associated secretory phenotype factors and enhances autophagic flux. These findings reshape our current understanding of the mechanisms underlying lysosomal/vacuolar (de-)acidification in both young and aged/senescent cells.

80B

Characterization of genetic mechanisms of antifungal peptide drug resistance in *Candida glabrata*

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Candidiasis is the most common human fungal infection, and as an opportunistic pathogen, *Candida* infections are particularly dangerous in immunocompromised patients. Four *Candida* species, including *Candida glabrata*, are listed by the World Health Organization among the top fungal pathogens posing the greatest threat to human health. *Candida glabrata* emergence as a major pathogen and its resistance to azoles and echinocandins presents serious therapeutic challenges. Histatins are antimicrobial peptides secreted by human salivary glands, among which histatin 5, a 24–amino acid peptide, shows potent fungicidal activity. Studies from our laboratory identified a small histatin inspired peptide, KM29 (Y-K-R-K-F-K-R-K-Y), that yields greater fungicidal activity than Histatin 5 against multiple *Candida* species. To explore the mechanism underlying this effect, previous studies in our laboratory used the *Saccharomyces cerevisiae* genome-wide deletion library to identify pathways influencing KM29 susceptibility. Resistant mutants were consistently linked to the mitochondrial electron transport chain, plasma membrane transporters, and sterol metabolism. Orthologous open reading frames

for sterol transporters *LAM1* and *SIP3* were retrieved from the Candida Genome Database, and CRISPR was used to create deletion mutations in *C. glabrata*. The resulting mutants were tested for susceptibility to KM29 using broth microdilution and MIC assays. *SIP3* deletion strains underwent further phenotypic characterization under exposure to common antifungal agents, as well as plasma membrane stressors. These experiments aim to clarify the role of sterol transport in KM29 resistance and may provide broader insights into antifungal resistance mechanisms in *C. glabrata*.

81B

Environment-dependent landscapes of coding variant impacts on coproporphyrinogen oxidase

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Hereditary coporphyria (HCP) — caused by variants in coproporphyrinogen oxidase (*CPOX*) — can be diagnosed via genome sequencing. However, 74% of clinically-reported *CPOX* missense variants are classified as variants of uncertain significance (VUS) due to lack of evidence. *CPOX* variant classification is further complicated by environment-dependence: For example, despite causing HCP in patients exposed to mercury, CPOX variant p.Asn272His (c.814A>C) has been classified as “benign”. Here we measured the functional impact of nearly all possible CPOX amino acid substitutions in both the presence and absence of mercury. The resulting CPOX variant effect maps reflect known protein structure and mutational tolerance patterns while also offering new sequence-structure-function insights. Scores from this atlas not only distinguish pathogenic from benign variants but also identify mercury-dependent variant impacts, thus informing our clinical, structural, and functional understanding of CPOX deficiency and illustrating the value of systematic context-dependent multiplexed assays of genetic variant effects.

82A

MMS21 allele incompatibility in *Saccharomyces* species

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Thermal niches are sharply partitioned among *Saccharomyces* yeasts, with thermotolerant *S. cerevisiae* and thermosensitive *S. uvarum* exhibiting distinct upper thermal limits. The genetic mechanisms underlying this divergence remain largely unknown. High-resolution chromosome mapping in interspecific hybrids revealed that orthologous alleles of *MMS21*, an essential SUMO E3 ligase, differentially affect thermotolerance. To measure the effects of *MMS21* divergence in the parental backgrounds, we generated allele replacements. We found that the thermosensitive *S. uvarum MMS21* allele caused loss of viability in *S. cerevisiae*, while the *S. cerevisiae MMS21* allele imposed a strong fitness defect in *S. uvarum*, indicating a reciprocal genome incompatibility. To further examine the effects on viability, we sporulated *S. cerevisiae* diploid heterozygous for *MMS21-S.c.* and *MMS21-S.u.* alleles. We found that all viable spores carried the *MMS21-S.c.* allele; however, segregation was not Mendelian. This defect in meiosis was rescued by providing *MMS21-S.c.* allele on a plasmid. To dissect the molecular determinants of this incompatibility and define their contribution to thermotolerance, we are employing a plasmid shuffle strategy with chimeric *MMS21* alleles to map regions of *MMS21* that underlie incompatibility and thermal sensitivity. We hypothesize that the *MMS21-S.u.* allele is incompatible with one or more *S. cerevisiae* proteins but remains viable and temperature-sensitive in the hybrid due to *S. uvarum* orthologs. These studies will establish how divergence in an essential SUMO E3 ligase contributes to both species-specific thermotolerance and species barriers across *Saccharomyces* species.

83A

Classroom experimental evolution reveals loss of paralog redundancy as a common mechanism of echinocandin resistance

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Antifungal resistance poses a growing threat to human health, yet the evolutionary pathways underlying resistance remain incompletely understood. We leveraged a robust experimental evolution framework to investigate adaptation of *S. cerevisiae* to echinocandin drugs. Across three academic years, 15 independent high school classrooms participating in the yEvo program evolved 199 populations via serial transfer in gradually increasing drug concentrations under slightly varying laboratory conditions. From each evolved population, resistant clones were isolated and subjected to whole-genome sequencing and variant calling. Mutations were detected in all sequenced isolates, with more than 50 genes recurrently mutated across independent samples. The most striking pattern, however, was the overwhelming prevalence of mutations in the partially redundant, synthetic lethal paralogs *FKS1* and *GSC2*, which encode catalytic subunits of the β-1,3-glucan synthase complex. Consistent with prior work, *FKS1* mutations clustered within three established hotspot regions. In contrast, despite the high level of sequence homology to *FKS1, GSC2* mutations predominantly appeared to be loss-of-function (LOF) variants. Notably, even though many samples had an *FKS1* mutation alone, nearly all samples with a *GSC2* mutation also carried an *FKS1* hotspot mutation, suggesting a strong epistatic interaction between the paralogs. We propose that loss of *GSC2* enhances resistance in the presence of an *FKS1* hotspot mutation, potentially by eliminating a functional paralog that would otherwise restore drug susceptibility. Supporting this hypothesis, experimental evolution conducted in an *fks1*Δ background resulted in the acquisition of homologous hotspot mutations in *GSC2* (as opposed to the LOF pattern seen in wild-type yeast). Phenotypic assays are now being conducted to determine the magnitude of resistance caused by these paralog mutations alone and in combination. The consistency of this result across heterogeneous classroom settings underscores the robustness of the genetic solution while also highlighting the power of distributed, education-integrated experimental evolution. Our findings reveal a previously underappreciated adaptive strategy for echinocandin resistance involving the coordinated alteration of paralogs and demonstrate how research and education can advance in tandem.

84A

How Do Population Size Differences Shape Genome Evolution over 7,500 Generations from SNPs to Karyotypes? Microscopic vs. Macroscopic Multicellularity in Yeast

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The transition from unicellular to macroscopic multicellular life represents a major evolutionary transition with profound consequences for population genetics. Multicellular organisms necessarily exist at lower population densities than their microscopic counterparts, fundamentally altering effective population size (Ne) and the balance between selection and drift. It is predicted that reduced Ne should weaken purifying selection, increase fixation of neutral and slightly deleterious mutations (elevated dN/dS), and reduce clonal interference, with the opposite effect happening when Ne is larger. However, testing this framework has been challenging, as comparisons across extant species confound population size with phylogeny, ecology, and mutation rate.

The Multicellularity Long-Term Evolution Experiment (MuLTEE) provides a unique experimental setting to test the effects of population size. From a single microscopic snowflake yeast ancestor (~30 µm radius), fifteen replicate populations evolved for >7,500 generations (1,500 days) under selection for large size and fast growth. Strikingly, ten populations evolving under oxygen limitation remained microscopic (~50 µm radius), while five populations where the oxygen constraint was lifted evolved macroscopic size (200 µm to mm scale, visible to the naked eye). This size divergence corresponds to a divergence in effective population size, macroscopic populations have a population size three orders of magnitude smaller than microscopic populations, allowing us to disentangle the effects of Ne from confounding variables.

To test how population size differences driven by multicellular size shape the selection/drift balance and generate distinct signatures of genome evolution, we sequenced isolates and populations from microscopic and macroscopic lineages at 3,000, 5,000, and 7,500 generations using both short- and long-read sequencing. First, by analyzing high-frequency SNPs, we found that macroscopic populations show 5 times more fixed or near-fixation mutations, whereas the SNP spectrum in microscopic populations is shaped by clonal interference. Next, building on our published 1,000-day work (Tong et al., 2025), we found that populations continue diverging at the ploidy and karyotype level through 1,500 days, and that macroscopic populations still sustain the specific aneuploidies accumulated during the microscopic-to-macroscopic transition. Finally, we explored structural variation in autosomes and mitochondrial genomes by combining short- and long-read genome data from microscopic and macroscopic strains. Together, this work demonstrates divergent signatures of genome evolution driven by differences in effective population size between microscopic and macroscopic multicellular yeast populations in a long-term evolution experiment.

85A

Environmental dependency of *de novo* gene evolution in yeast *Saccharomyces cerevisiae*

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Novel protein-coding genes can emerge *de novo* from ancestrally noncoding sequences in many species, including yeasts, and promote adaptation to environmental stresses. Previous work proposes that pervasive translation of lowly expressed open reading frames (ORFs) in noncoding regions creates a rich reservoir of “proto-genes”, whose subsequent acquisition of gene-like properties during evolution, such as increased expression, may be favored or purged by natural selection depending on their phenotypic impact. However, whether and how environmental stresses affect the phenotypic consequences of proto-gene evolution remains unclear. In this study, we used *Saccharomyces cerevisiae* as a model to experimentally simulate proto-gene evolution through overexpression and systematically examined its impact on growth phenotype across stress and control environments. We individually overexpressed ORFs with prior evidence of native translation in *S. cerevisiae*, including 937 *de novo* ORFs and 4,036 conserved ORFs, making this dataset the largest of its kind in any species. High-throughput phenotyping revealed that the growth effects of *de novo* ORF overexpression varied strongly across environments. Gene-by-environment interaction analyses showed that 12.4% and 4.4% of *de novo* ORFs exhibited overexpression phenotypes modified by the stressors NaCl and tunicamycin, which induce osmotic and endoplasmic reticulum stress, respectively. Notably, among these stress-responsive *de novo* ORFs, stressors more often improved their overexpression phenotypes, by enhancing beneficial impacts or reducing deleterious ones, than worsened them. A follow-up screen across 22 diverse environments revealed that the average impact of *de novo* ORF overexpression on growth became less deleterious or more beneficial, as stress severity increased. Widespread phenotypic tradeoffs were observed, with 84% of *de novo* ORFs that conferred growth benefits in at least one environment also producing deleterious effects in at least another. Together, these findings demonstrate that both the direction and magnitude of the growth effects of *de novo* ORF overexpression are highly environment-dependent in *S. cerevisiae* and suggest that stressful environments may be more permissive to proto-gene evolution.

86A

The Extent of Genetic Incompatibilities Between Distantly Related Yeast Species

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Genetic incompatibilities arise when genetic differences between populations cause hybrid offspring to be inviable or sterile, making them one of the fundamental forces driving evolution and speciation. Traditionally, studying these incompatibilities has required crossing related species and dissecting the genetics of the resulting hybrids, which inherently limits the scope of the work to organisms that can still interbreed. Synthetic genomics opens a very different path: instead of relying on natural hybrids, we can build them. In our lab, we are applying this idea through a strategy we call collinearization, in which we artificially reconstruct the gene order of one species within the genome of another, bypassing major reproductive barriers such as the lack of synteny. Using this approach, we previously replaced one sixth of Saccharomyces cerevisiae Chromosome I with collinearized sequences from the distantly related yeast Kluyveromyces marxianus, revealing that ~12% of examined genes encode proteins that are incompatible between these species. We have now taken the next step and nearly completed an entire S. cerevisiae Chromosome I containing collinearized coding sequences from K. marxianus via a hierarchical assembly approach. This synthetic chromosome will be sequence verified and we will use global transposon mutagenesis to systematically uncover additional incompatibilities between these two species. I will present our current progress in this work, which will reveal the extent of genetic incompatibilities between two distantly related species.

87A

Evolutionary adaptation proceeds through a small number of phenotypic modules

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Understanding how the myriad molecular impacts of mutation percolate to influence higher-order traits and ultimately fitness requires compressing a many-to-many mapping into something tractable. Decades of theoretical work suggest this may be possible because biological systems are modular: effects of perturbation are often funneled through particular pathways or subsystems rather than propagating freely through the organism. Yet few empirical systems have been able to demonstrate such modularity at scale. Prior low-dimensional models hinted that mutation–fitness relationships can collapse onto a small number of latent axes, but these studies relied on simpler datasets, leaving open whether such structure generalizes to more genetically and environmentally complex systems. Here we show that, even across 774 diverse yeast lineages, fitness variation across 12 drug environments is organized by a strikingly low-dimensional structure defined by only a few inferred phenotypic axes that capture the main patterns of variation. Consistent with these lineages having evolved under strong selection pressure, they reveal a striking asymmetry in the genotype–phenotype map: their mutations exhibit broad pleiotropy, affecting nearly all inferred phenotypic axes, yet fitness in any given drug depends on a much sparser subset of the phenotypic modules these axes reflect. This architecture aligns with central expectations of evolutionary theory. Strong selection often favors mutations with broad physiological effects, whereas long evolutionary history shapes organisms into modular systems in which only certain trait combinations matter to fitness in particular environments. By compressing many-to-many relationships, this low-dimensional framework exposes the modular fitness space that constrains the pleiotropic effects of adaptive mutants. It also lays the groundwork for identifying the key phenotypic modules that matter for fitness across different environments.

88A

Recreating the b-h fusion of SAR lineage in budding yeast restores function of native subunits in ATP synthase.

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ATP synthase is a mitochondrial enzyme complex composed of multiple subunits and essential for ATP production. Subunits b and h form part of the stator stalk, a structural component linking the catalytic and membrane domains. In the SAR supergroup (Stramenopiles, Alveolates, Rhizaria), comparative genomics suggests that b and h fused into a single nuclear-encoded gene ~one billion years ago. To investigate the functional implications of this evolutionary event, we constructed and verified a fusion of the b and h sequences (b–h fusion) in *Saccharomyces cerevisiae*, where the two subunits are normally encoded separately. We show that the b-h fusion protein restores respiratory growth in yeast lacking the native h subunit. Structural modeling with AlphaFold indicates minimal change in the assembly of the stator stalk with the fusion. These results support a neutral evolutionary process rather than an adaptive one as the explanation of the b–h fusion in SAR.

89A

Mapping history dependence along evolutionary trajectories

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Evolutionary trajectories often follow complex paths through genotype–phenotype landscapes. When initiated from identical starting conditions, replicate populations fix different mutations, but it remains unclear whether such trajectories are functionally interchangeable. In particular, it remains unclear whether populations that reach similar fitness through different mutational paths occupy comparable phenotypic states, or instead represent distinct evolutionary solutions. Here, we address this question using high-throughput CRISPR-based methods to introduce large numbers of targeted genomic perturbations. We evolved many parallel *Saccharomyces cerevisiae* populations under identical conditions and systematically reintroduced mutations that naturally arose during these trajectories both into their original line and into other replicate lines. This approach allows us to measure the effects of real mutations that accumulated during evolution across diverse genetic backgrounds and at multiple evolutionary stages. By quantifying how the consequences of the same mutation depend on where and when it occurs, we reveal the structure of functional divergence among trajectories and track how local genotype–phenotype landscapes change over the course of evolution.

90A

High-throughput technique to assay mutation spectra in S. *cerevisiae* natural isolates

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Mutations create genetic variation and drive evolution, yet a species-wide understanding of how mutation rates and spectra vary within natural populations remains lacking. Evolutionary theory often assumes a constant mutation rate, however, recent evidence suggests that mutation processes can differ among individuals and populations. Within-species variation in mutation rates and spectra can reveal how mutation rates may have evolved in nature. However, due to the low occurrence of such a rate under normal conditions, current methods, such as mutation accumulation (MA) or reporter-based fluctuation assays, cannot measure mutation rates at large scale, leaving it an open question.

To address this challenge, we are developing a high throughput genomic technique to characterize mutation rate and spectrum variation across natural isolates of *Saccharomyces cerevisiae*. By combining pooled growth, a reporter-gene assay, and long-read sequencing with PacBio HiFi, this approach links de novo mutations to naturally occurring single nucleotide polymorphisms (SNPs) that serve as intrinsic barcodes. We have amplified an 8 kb region flanking the *CAN1* reporter gene across diverse natural isolates; polymorphisms within this region enable strain identification without added barcodes. This design enables multiplexing of hundreds of natural isolates in a cost-effective manner. Preliminary results show that the PacBio HiFi approach recovers mutation spectra consistent with those obtained using Illumina short-read sequencing, demonstrating technical feasibility and reliability.

Ongoing work focuses on scaling the method and validating strain-specific mutation spectrum. We are currently optimizing key steps of the library preparation to ensure robust recovery and indexing of mutant samples. Upon completion of this optimization, we will conduct an initial long-read sequencing

run to evaluate the mutations, validate the correct assignment of mutations with respect to their genetic background and assess whether strains are proportionally represented in the pool. These experiments will serve as a critical proof of principle for the scalability of and reliability of the approach before broader implementation across more natural isolates. By enabling species-wide comparisons of mutation rates and spectra, this method provides a foundation for identifying natural mutator alleles. More broadly, it advances understanding of how genetic variation shapes mutational processes within a species.

91B

Gastrointestinal evolution of *Saccharomyces 'boulardii'* probiotic yeast in germ free mouse model

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Saccharomyces cerevisiae var. '*boulardii*' holds promise as a powerful therapeutic tool as probiotic and chassis. However, immunocompromised patients are at risk of bloodstream infections, including infections caused by probiotics. Therefore, addressing microbiome dysbiosis by live therapeutics in a manner that is safe to patients is challenging. Recently we demonstrated that the combination of *in vivo* virulence assessment and stress phenotyping is an efficient approach to determine virulence factors in *S. 'boulardii'* isolates. By deleting a single gene, we showed that the probiotic yeast can be engineered to exhibit reduced virulence, making it a promising, safe probiotic option for patients with weakened immune system. However, information about gut evolution of *S. 'boulardii'* and its genetically modified versions is scarce, hindering their clinical application. Genetic modifications might increase the chance of developing complex phenotypes and could compromise the native and engineered probiotic functions. In our present work we applied *in vivo* experimental evolution, combined with a comparative genomics approach and stress phenotyping to identify adaptation mechanisms of the yeast probiotic strain *S. 'boulardii'* MYA-796 in the gastrointestinal tract of germ-free C57BL/6 mice. Gastrointestinal colonization was followed for 6 weeks; yeast colonies were isolated from fecal material on the 1st, 3rd, 5th and 6th week and used for whole genome sequencing. Our results showed that chromosome XV trisomy among the isolates become prevalent by week 6. Evaluation of SNPs and INDELs in the single colony isolates revealed that 19 genes were affected by in total 37 high effect mutations. Additionally decreased tolerance to Congo red, Amphotericin B and NaCl, as well as increased invasivity both on nutrient rich and nutrient deprived agar plates. The trisomy of chromosome XV and the observed mutations and phenotypes suggest rapid adaptation to heat stress, changes in cell wall composition, increased sensitivity to osmotic stress and adaptation to nutrient-limited environments. These results demonstrate that *S. 'boulardii'* may acquire genetic traits during gastrointestinal colonization, leading to the emergence of complex phenotypes. We show that gastrointestinal evolution of *S. 'boulardii'* probiotic yeast is an important phenomenon, that needs to be considered when application of genetically modified strains is considered.

92B

Yeast 'survivor' game: predicting long-term evolutionary success from short-term fitness

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In this study, we investigate the relative contribution of initial fitness to the long-term success of a genotype competing in a naturally diverse population. Specifically, we compete over 300 genetically barcoded *S. cerevisiae* isolates in a pooled setting for over 700 generations. We found that the strains that remain at detectable frequency until the end of the competition uniformly come from the top 95th percentile in the initial fitness values, making initial fitness the most significant predictor of long-term success. However, we occasionally see heterogeneity in the competition outcomes, which suggests a role of stochastic adaptation, and clonal interference. We demonstrate that the "finalists" of our competition change on the genetic level, and that the spectrum of *de novo* mutations depends both on the strains' genotype and environment. Finally, we show that gene targets of the novel mutations are specific to the combination of strain identity and environment, even among the genetically similar strains and environments that select for the same strains in the beginning of the competition.

93B

Apoptosis upon diverse cellular stresses in yeast

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Across all domains of life, microbial species have been observed to perform genetically-encoded, apoptosis-like cell death. Ancient microbes encoded apoptotic factors, which have been maintained within extant species over approximately 1.8 billion years. Despite this broad conservation, long-standing questions about how apoptosis provides an adaptive advantage during microbial evolution remain unanswered. One enduring obstacle remains our lack of a systematic understanding about the cellular stresses that trigger apoptosis in microbes. To address this question, we screened all possible single-gene perturbations in Saccharomyces cerevisiae to identify cellular stresses that trigger apoptosis via that sole yeast caspase homolog, YCA1. Gene knockdowns that trigger YCA1-dependent apoptosis are expected to cause a decrease in survival that is, in turn, partially or fully rescued by YCA1 deletion. To identify such genetic interactions, we performed a CRISPRi screen for knockdowns that decrease survival in a YCA1-dependent manner, using barcode frequencies as the screen readout. Analysis of our screen hits reveals that YCA1-dependent apoptosis is triggered by disruptions to several cellular processes, including: RNA processing, ribosomal biogenesis, chromatin organization, mitotic cell cycle, and mitochondrion organization. Experimental work is underway to further elucidate (1) how perturbations to each process trigger the apoptotic cascade and (2) how evolutionary dynamics are shaped in the presence vs. absence of the apoptotic response.

94B

Identifying key residues which determine receptor use of human alphacoronaviruses 229E and NL63 using chimeric spike proteins

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There are seven known coronaviruses that infect humans; four that cause the common cold and three that recently caused epidemics, including SARS-CoV-2, the virus responsible for the COVID-19 pandemic. While the common cold coronaviruses have been circulating in humans for decades, little is

known about their evolutionary history and relations. Two of these viruses, human alphacoronaviruses (HCoVs) 229E and NL63, have closely related spike proteins yet bind to different human receptors for cellular entry to initiate infection. HCoV-229E binds aminopeptidase N (APN) and HCoV-NL63 binds angiotensin-converting enzyme 2 (ACE2). The receptor binding motifs (RBMs), where these two viruses contact their receptors, are approximately 60% diverged.

To understand which of these residues are essential for binding and receptor specificity, we will make chimeric spike proteins from the two viruses and test their receptor binding using a yeast display assay. Yeast are an ideal model system for this study because we can express many chimeric spike proteins and run the assay to measure their binding simultaneously. Because the three RBMs are contained in loops, we will first swap the whole RBM loops to create all possible combinations in a constant backbone. These loop swap chimeric spikes will then be expressed in on the yeast cell surface with either the ACE2 or APN receptor added to the solution, and the binding ability will be measured using flow cytometry.

To determine which specific amino acid residues are key for binding, we will make a library of chimeric spike proteins which will encompass thousands of possible combinations of amino acids from the individual positions where HCoV-229E and HCoV-NL63 differ in the RBM loops. We will then use the same expression assay to measure binding, followed by next-generation sequencing of the pooled well-binding chimeras to identify sites required for binding. This will also give us insight into how difficult it would be for these viruses to hypothetically switch which receptor they bind to.

95B

Low-fitness yeast benefit from mutation accumulation in some environments

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Mutations have worse effects in yeast genetic backgrounds with high fitness. One recent model even predicts that the effects of a class of engineered mutations can become net positive below a 'pivot' background growth rate. The existence of 'pivot' growth rates in the range of positive growth seems to contradict the fact that mutations should have deleterious effects on average and would predict that yeast below the pivot growth rate should improve with mutations even in the absence of selection. To test this, we performed a mutation accumulation experiment with 20 lines for each of 20 founder backgrounds, accumulating an average of six mutations per line, and compared growth rates of the 400 lines between the beginning and end of the experiment. In rich media, we find evidence for the existence of a pivot growth rate, with the three lowest-fitness founders improving on average. However, in a pH-buffered synthetic media, we do not observe any correlation between mutational effects and founder background growth rate.

96B

A physiological basis for history-dependent fitness effects in natural *S. cerevisiae* isolates

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Environmental change is ubiquitous in natural settings, requiring organisms to adapt to multiple environments. Furthermore, we have previously shown that environmental fluctuations also pose the novel challenge of adaptation to rapid environmental transitions. In contrast to experimental evolution which proceeds via a small number of large-effect mutations, natural variation encompasses many genomic changes that accumulate over millions of years. Whether findings from lab-based evolution experiments, such as ours, can be generalized to natural variants – despite these differences – remains an open question. Here, we ask how environmental fluctuations affect the fitness of a collection of ~300 DNA-barcoded natural *S. cerevisiae* isolates across ~30 environments. We find that overall fitness in fluctuating environments cannot be fully predicted by fitness in static environments. Additionally, we observe even larger fitness effects in the component environments, than expected based on deviations from overall fitness predictions. We develop a simple physiology-based mathematical model that recapitulates our observations. The remarkable consistency between the behavior of natural isolates and experimental evolution-derived mutant strains suggests a universal mechanism underlying the impact of environmental change on fitness.

97B

The Role of Structural Variants in Domestication of *Saccharomyces cerevisiae* to Baking Environments

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The species *Saccharomyces cerevisiae* is associated with many human associated environments such as bakeries, breweries, distilleries, and wineries. The evolutionary effects of domestication of *S. cerevisiae* to these environments have been a major focus of recent research. Studies have found evolution of *S. cerevisiae* strains to be driven by both location and ecological niche, and show signs of domestication such as higher ploidy level, aneuploidy, increased gene copy number variation (particularly in loci in subtelomeric regions), and genome decay. An increasingly interesting sign of domestication is the presence of large structural variants. It has been found that structural variants are more frequent in strains exhibiting higher ploidy level and aneuploidy, are enriched in subtelomeric regions, and are associated with particular phenotypes. However, how structural variants are related to adaptation to particular human associated environments requires further attention. Here, we leverage a collection of baking associated strains which vary by geography, isolation source, ploidy level, and presence of aneuploid chromosomes as a model of domestication. Using this collection, we assembled phased genomes and identified structural variants in relation to genomic features. This study will ultimately help elucidate the role of structural variants in strains that have undergone domestication.

98B

The path of yeast resistance: drug resistance via aneuploidy in Saccharomyces cerevisiae

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Copy number variants (CNVs) of ERG11 are known to play a major role in acquisition of resistance to the commonly used azole family of antifungal drugs in many pathogenic yeast species. However, the precise conditions in which these CNVs arise, and what affects their dynamics, have not been

systematically studied. Here, we use a fluorescent reporter to track CNVs at the ERG11 locus in Saccharomyces cerevisiae, to study the effect of different concentrations of fluconazole (strength of selection), growth temperature, and of background ploidy, on CNV formation.

We found that ERG11 CNVs emerge reproducibly at ~MIC and sub-MIC concentrations. With increase in temperature, CNV emergence was generally more rapid, and they had a greater tendency towards fixation. ERG11 CNVs were also selected for in diploids, but predominantly at one concentration only, and with no clear temperature effect. Unexpectedly, all the evolved CNVs were revealed to be full-chromosome aneuploidies. This tendency towards aneuploidy is independent of the genomic location of ERG11, and instead appears to be a characteristic of adaptation to fluconazole. The evolved CNV strains provided a significant growth benefit at particular fluconazole concentrations, and we show that this growth benefit can be explained primarily by the extra copy of the ERG11 gene. Overall, we find that ERG11 CNVs facilitate rapid adaptation and provide a selective advantage, within a narrow range of selective conditions.

99B

Resolving the aneuploidy paradox by experimental evolution

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Aneuploidy, characterized by unbalanced chromosome numbers, is commonly associated with reduced fitness, such as slow growth and poor viability. Paradoxically, it is also a classic hallmark of cancer, contributing to tumorigenesis and treatment resistance. The mechanisms underlying this apparent "aneuploidy paradox" remain largely unknown. Here we investigated the evolutionary dynamics of genomic, transcriptomic and phenotypic alterations of a systematically constructed panel of yeast aneuploidies (1N+1 and 2N+1) under scenarios of neutral and adaptive evolution respectively. Across ~3000 generations of neutral evolution, almost all constructed extra chromosomes were stably inherited, rather than returning to euploidy given the reduced fitness. The 2N+1 aneuploidies revealed higher rates of chromosome copy number alterations compared to euploid diploid and such rates keep increasing as evolution goes on, suggesting the existence of extra chromosomes fuels chromosome instability. Dosage compensation, whereby transcript abundance is adjusted to compensate for altered gene copy number, is almost absent for chromosome gains but exists for chromosome losses. The SNV and INDEL rates are comparable between aneuploidies and their euploid counterparts. Under adaptive evolution, several constructed chromosome gains confer fitness advantages to specific stresses, which was further supported by recurrent acquisitions of the same chromosome in other populations initially not carrying this chromosome, indicating chromosome-specific advantages of aneuploidies in the selected environments. Moreover, aneuploidy drives the initially clonal populations to become increasingly heterogeneous, providing abundant genetic variation for selection to act upon towards adaptation. Taken together, through distinct experimental evolution settings, we comprehensively characterized the evolutionary trajectories of aneuploidies with different karyotypes and elucidated the multi-layered molecular basis underlying the aneuploidy paradox.

100B

The Hidden Life of Yeast Viruses

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In recent years, sequencing studies have revealed a vast array of RNA viruses that infect many fungal species. These mycoviruses are well known to persist as endemic infections transmitted through cell division or fusion with no known extracellular route. Although often believed to be asymptomatic, mycoviruses are now understood to profoundly influence fungal phenotypes in highly consequential but poorly understood ways. Prominent examples include mycoviruses that induce hypo- or hyper-virulence in fungal pathogens that infect plants, insects, and humans. Thus, mycoviruses are better understood as spanning the symbiotic spectrum, contributing to extra-chromosomal 'missing heritability' that shapes fungal phenotypes. Here we investigate mycovirus controlled phenotypes at unprecedented scale using the budding yeast *Saccharomyces cerevisiae*. We utilize a collection of 1,011 yeast isolates from across the planet, each with a sequenced genome, transcriptome, and comprehensive phenotypic profile. Through computational analysis of their transcriptomes, we establish the yeast RNA virome across the entire collection, including 14 new mycoviruses in yeast. Leveraging this new resource, we discover a robust virome-phenome association, including a particular role of mycovirus infection for progression through the sexual cycle (sporulation) as well as new viral encoded toxins and a novel toxin defense system. These findings reveal the tremendous potential of yeast for investigating mycovirus phenotypes, opening this burgeoning field to the unique strengths of this model organism.

101A

Exploring the Impact of H2A.Z Depletion on Transcription Regulation

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H2A.Z is an evolutionarily conserved histone variant. In *S. cerevisiae*, it is incorporated into chromatin by the SWR1 chromatin-remodeling complex. While implicated in transcriptional regulation, among many other processes, H2A.Z's exact role in this process is still debated, as gene expression does not appear to correlate with its localization at gene promoters. The limited transcriptional alterations observed in previous studies may stem from experimental design factors such as the use of knockout models and nutrient-rich growth conditions. These may overlook compensatory mechanisms and H2A.Z's role in gene regulation, particularly in priming genes for transcription under specific environmental conditions. To address these limitations, I used the Anchor-Away system to deplete H2A.Z. Using whole-transcriptome sequencing across three strains (wild-type, H2A.Z deletion, and Anchor-Away depletion), I aim to reveal the immediate effects of H2A.Z loss and uncover previously undetected changes in gene expression to explore H2A.Z's role in transcriptional regulation. This current dataset serves as a foundation for future analyses that will integrate stress induction (e.g., caffeine treatment) to investigate how H2A.Z contributes to the response to cellular stress. Overall, this project will dissect the role of H2A.Z in transcriptional regulation, particularly under stress

conditions, contributing to our understanding of its impact on eukaryotic cellular processes and helping resolve the debate surrounding H2A.Z's role in transcription regulation.

102A

Investigating a prion-like form of the mRNA cap methyltransferase

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Prions are alternate structures of proteins that are propagated across generations by self-templating their structural forms, in a distinctive form of epigenetic inheritance. Prions exist in many domains of life, including mammals, plants, fungi, bacteria, and viruses. Proteome-wide screens in budding yeast revealed that dozens of proteins can form prions, some providing adaptive advantages under stress. Despite their prevalence, prions remain an understudied mode of epigenetic inheritance. Of particular interest to our lab are prions that may regulate RNA. Through a large phenotypic screen in budding yeast, testing dozens of RNA modification enzymes, we discovered that the gene ABD1, encoding the mRNA cap methyltransferase, when transiently overexpressed under stress could precipitate a novel example of a prion-like form. Abd1 is an essential enzyme whose function is conserved across all eukaryotes. It transfers a methyl group from S-adenosylmethionine (SAM) to the N7 amine of the 5´ guanosine cap on mRNA. The prion-like form of Abd1 (that we denote as [*ABD1*$^{+}$]) promotes resistance to translation inhibition by cycloheximide compared to a naïve strain. This trait exhibits genetic characteristics of a prion – it is mitotically stable through hundreds of generations of yeast outgrowth, shows dominant inheritance through meiosis, and is dependent on the chaperone Hsp70. [*ABD1*$^{+}$] strains are also resistant to growth inhibition by the antifungal drug sinefungin, a competitive inhibitor of Abd1, as compared to naïve cells. This suggests that Abd1 may have an altered structure in its prion-like state, and thus may have altered catalytic activity. In ongoing work, we are exploring sequence features of Abd1 that are important for its prion-like phenotypes. Additionally, we are interested in transcriptome-wide consequences of the prion-like state that may provide clues of how [*ABD1*$^{+}$] phenotypes are expressed. Overall, our work contributes a new example of how an enzyme essential for the mRNA lifecycle might have an altered function through an epigenetic, heritable state.

103A

Translational control of CAK and Cdk T-loop phosphorylation in response to growth in yeast

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Cyclin-dependent kinases (Cdks) require activating T-loop phosphorylation, a modification that is considered constitutive. Here, we examined the regulation of the Cdk-activating kinase, Cak1, in budding yeast. We measured Cak1 levels and the activating T169 phosphorylation of Cdc28 (the budding yeast Cdk) across various nutrient environments. We found that the abundance of Cak1 and the T169 phosphorylation is significantly reduced in cells that are proliferating very slowly or have entered quiescence. A small upstream open reading frame (uORF) in the CAK1 transcript represses Cak1 synthesis, especially in poor growth conditions. Eliminating the uORF increased Cak1 levels but did not alter proliferation kinetics under most laboratory contexts. Instead, it reduced the viability of quiescent cells and the fitness of slowly proliferating chemostat cultures. In cells lacking several type 2C protein phosphatases, which remove the T169 phosphorylation, there was a pronounced acceleration of initiation of cell division in the absence of the uORF in CAK1. Our results suggest an unexpected layer of control, impinging on the activating phosphorylation of the Cdk. The uORF-mediated repression of Cak1 synthesis directly couples protein synthesis to the activity of the core cell cycle machinery.

104A

A role for the RNAPII phosphatase, Fcp1, in regulating Rpb1 protein levels

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Rpb1, the catalytic subunit of RNA Polymerase II (RNAPII), contains a highly conserved C-terminal domain (CTD) that can be differentially phosphorylated during the various stages of transcription. The essential and dynamic phosphorylation state of the CTD is maintained in *S. cerevisiae* by several kinases and phosphatases, including Cdk8 and Fcp1. Previously, we observed that slow-growth phenotypes caused by the FCP1 truncation (*fcp1-594*) were suppressed by loss of CDK8, suggesting there is a functional relationship between these two CTD-modifying enzymes that remains to be fully elucidated. To determine if the link between Fcp1 and Cdk8 involves a shared activity on the RNAPII-CTD we measured the total levels of RNAPII subunits in wild type, *cdk8*Δ, *fcp1-594*Δ, and *cdk8*Δ *fcp1-594*Δ mutants. We found that the *fcp1-594* mutant lead to elevated Rpb1 levels, while other CTD phosphatases mutants (*ssu72-2* and *rtr1*Δ) did not. We then utilized public databases to find transcription factors that have shown DNA binding and expression, and found that Rpn4 was the only RNAPII subunit transcription factor that uniquely targets RPB1 promoters. Interrogating this relationship further, we found that the loss of both RPN4 and CDK8 normalized the elevated Rpb1 levels caused by the FCP1 truncation. Together, these findings uncovered a novel role for Fcp1 in Rpb1 biology and expanded the Rpb1 regulatory network to include Cdk8 and Rpn4, thereby providing new insight into the mechanisms that govern RNAPII homeostasis.

105A

Regulation of the conserved TATA-Binding Protein-Associated Factor 2 abundance by the Ubiquitin-Proteasome System

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The general transcription factor TFIID is essential for accurate transcription initiation by RNA polymerase II and is composed of the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs). Among these, TAF2 plays a crucial role in stabilizing TFIID binding to the core promoter and is highly conserved from yeast to humans. Misregulation of TAF2 has been linked to several cellular pathologies, including Microcephaly Thin Corpus Callosum Intellectual Disability Syndrome and congenital heart disease. Notably, TAF2 expression is upregulated in hepatocellular carcinoma and high-grade serous ovarian cancer, though the molecular basis for this dysregulation remains unknown. To investigate potential mechanisms, we tested whether the ubiquitin-proteasome system (UPS) regulates the cellular abundance of TAF2. Using *Saccharomyces cerevisiae* as a model, we found that TAF2 undergoes

polyubiquitination and degradation via the 26S proteasome, revealing UPS-mediated control of this evolutionarily conserved transcription factor. These findings suggest that proteasomal turnover of TAF2 may be critical for maintaining optimal TFIID composition and that disrupted UPS regulation may contribute to TAF2 upregulation in cancer.

106A

Regulatory control of the yeast homolog of human gene defective in the juvenile form of Batten disease

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The juvenile form of Batten disease is a progressive neurodegenerative disorder caused by mutations in the gene ceroid lipofuscinosis, neuronal 3 (CLN3). Although CLN3 is highly conserved across eukaryotes, its cellular function and the mechanisms that control its expression remain unclear. To bridge this gap, we studied the expression of the *S. cerevisiae* homolog, YHC3/BTN1, using a HIS3 reporter to identify cis and trans genomic elements that regulate YHC3 expression. There are five upstream open reading frames (uORFs) in YHC3 at -180, -96, -80, -66, and -27. Mutagenesis of the AUGs in the uORFs to AAG increased the expression of the HIS3 reporter, demonstrating that one or more uORFs repress translation of YHC3. This is further evidenced by mutagenesis of three stop codons at -18, -9, and -3 from UAG to UUG (-18) and UAA to UUA (-9 and -3) within the uORF most proximal to the main YHC3 coding sequence. Mutagenesis of these stop codons increased the expression of the HIS3 reporter, but to a lesser extent than augmenting the AUGs to AAG, further supporting the idea that multiple uORFs repress translation of YHC3. 5' RACE indicates that two or more of the uORFs are contained in the mRNA. Additionally, we have identified genes that, when expressed from the high-copy vector, YEp13, increase reporter expression. Examples of two of these genes, WHI2 and GCN3, suggest involvement of pathways in the general stress response and the amino acid-sensing pathway. This suggests that YHC3 expression responds to amino acid limitation and is involved in TORC1-related pathways. Overall, our data support a model in which YHC3 is controlled by TORC1 signaling and amino acid-sensing pathways, with translational control mediated by inhibitory uORFs. This nutrient-responsive regulation suggests that YHC3 may play a role in cellular adaptation to metabolic stress. Supported by grants from NIH, R35GM131731, and from the ForeBatten Foundation.

107B

Deriving functional insights into RNA polymerase II transcription elongation through evolutionary analyses and suppressor genetics

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In eukaryotes, transcription through chromatin requires the functions of regulatory factors that promote the elongation rate and processivity of RNA polymerase II (RNAPII). Among these transcription elongation factors (TEFs), the Polymerase Associated Factor 1 complex (Paf1C) also directly stimulates co-transcriptional histone modifications, such as the mono-ubiquitylation of lysine 123 on histone H2B in yeast (H2Bub). Because Paf1C and other TEFs have been almost exclusively studied in a few model organisms, the extent to which their functions are conserved across species remains relatively unexplored. Recently, we utilized a domain-centric Hidden-Markov Model (HMM) based pipeline to identify putative homologs of the five Paf1C subunits and five other TEFs in 304 species across the Tree of Life. We found that Paf1C homologs are only detected in eukaryotes, whereas the TEFs Spt4, Spt5, and Elf1 are also detected in prokaryotes. Residues in Paf1C that interact with components of the RNAPII elongation complex are conserved, and Paf1C domains, like the Rtf1 histone modification domain (HMD) that stimulates H2Bub, are detected in most eukaryotes with some notable exceptions. These observations indicate that Paf1C's roles in promoting RNAPII activity and H2Bub deposition are broadly conserved. Previous studies showed that Paf1C mutants exhibit transcriptional defects and attenuated H2Bub levels. H2Bub is critical for maintaining chromatin structure, is a prerequisite for other histone modifications, and is associated with human cancers. It is unclear which transcriptional defects observed in the absence of Paf1C can be directly attributed to H2Bub loss. To differentiate the contributions of H2Bub from other Paf1C-dependent functions, we have developed an induction system to recover H2Bub in *S. cerevisiae* strains lacking a functional Paf1C. We are utilizing time-resolved genomic approaches to test if nascent transcription and RNAPII occupancy defects are rescued upon H2Bub recovery in Paf1C mutants. Our study is exploring how conserved TEFs contribute to gene expression regulation and to what extent H2Bub, a histone modification long associated with transcription, is directly involved in regulating the process *in vivo*.

108B

Mapping MATalpha1 transcription factor residues that determine binding site

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The HMG-box family, an abundant and ubiquitous protein family, contains various DNA-binding proteins that bind differing DNA sequences. Identifying the differences in amino acid sequences that cause such divergence in DNA binding sites could provide insights in transcriptional regulation and may also have implications in computational models that assess disease risk of genetic variants in transcription factors. Although these proteins are widely found in eukaryotes, our understanding of the determinants of HMG-box sequence specificity remains unclear. Therefore, we set out to identify the cause of the differential DNA-binding motifs of MATalpha1 between *Saccharomyces cerevisiae* and *Candida albicans* to help our understanding of HMG-box target specificity.

Baker, et al., 2011 previously studied the divergence in MATalpha1 binding site preference for *S. cerevisiae* and *C. albicans* using vectors with species-specific binding sites upstream of a beta-galactosidase reporter that were activated by MATalpha1 homologs expressed in *S. cerevisiae*. To allow high-throughput screening, we have replaced the beta-galactosidase reporter with GFP. We will create a plasmid library containing around 10,000 chimeric MATalpha1 genes using large-scale oligonucleotide synthesis. We will use fluorescence-activated cell sorting to isolate cells with chimeric MATalpha1 proteins that either can or cannot utilize each species-specific MATalpha1 binding site, and then determine which plasmids were enriched in each pool using Illumina sequencing. Armed with the binding ability of each chimera, we will use a QTL-mapping analytical framework to identify the specific residues important for determining MATalpha1 binding-site preference in *S. cerevisiae* and *C. albicans* as well as characterizing the genetic interactions between these identified residues. We are currently creating the chimeras for analysis.

109B

Genetic Regulation of a Novel Interspecies Interaction Between *Candida albicans* and Anaerobic Bacteria

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The human gastrointestinal (GI) tract is inhabited by a wide variety of microorganisms. *Candida albicans* is one of the most common fungal pathogens of humans that can cause superficial mucosal infections and severe disseminated infections, especially in immunocompromised individuals. Biofilm formation provides *C. albicans* with a unique protective environment that allows this fungal pathogen to evade the host immune response and protects the fungus from antifungal drugs. We have shown that when *C. albicans* is grown with certain strictly anaerobic bacterial pathogens (e.g., *Clostridium perfringens* and *Bacteroides fragilis*), under aerobic culture conditions, these bacteria are able to induce *C. albicans* to form a novel interspecies interaction phenotype called a “mini-biofilm”. Mini-biofilms are free-floating, biofilm-like, cellular aggregates that form under planktonic (suspension) conditions only when *C. albicans* is cultured with these anaerobic bacteria or with cell-free spent-medium from the anaerobic bacterial cultures. The regulatory processes involved in the formation of mini-biofilms are not known. Complex biological processes are often regulated by a network of transcription factors (TFs) controlling a subset of target genes. Here, we investigate the transcriptional regulation of *C. albicans* mini-biofilms that form in the presence of *C. perfringens*, which is also a clinically relevant colonizer of the GI tract. Using a combination of genome-wide RNA-seq and CUT&RUN approaches, we identified a set of 14 TFs that govern mini-biofilm formation. These 14 TFs regulate over 2,600 downstream target genes to govern the process of mini-biofilm formation.

110B

CLN3 Translational Efficiency and Global Protein Synthesis During the Yeast Cell Cycle

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Entry into the cell cycle in S. cerevisiae is triggered by the most upstream G1 cyclin, Cln3p. Whether the abundance of Cln3p is dynamic during the cell cycle and, if so, how it is regulated have been matters of debate. Here, we show that the translational efficiency of *CLN3* changes in the cell cycle, peaking in the G1 phase, accompanied by changes in Cln3p levels. Pulse-chase analysis showed that these changes mirror a global, cell cycle-dependent oscillation in the bulk rate of protein synthesis.

Our data suggest that removing a 5' upstream Open Reading Frame (uORF) in *CLN3* reduces the range of Cln3p levels during G1. To examine how the cell modulates global synthesis rates despite constant ribosome abundance, we analyzed the ribosomal proteome during the cell cycle. We found specific post-translational modifications (PTMs) that fluctuate in abundance, potentially explaining the changes in ribosomal activity. Our results support a model in which modifications to the translation machinery produce global protein synthesis waves during the cell cycle, unequally affecting the translational efficiency of certain transcripts, including *CLN3*. As a result, Cln3p abundance rises in G1, promoting the G1/S transition.

111B

Unraveling the distinct roles of the alpha-like subunits in RNA polymerase I and III complex biogenesis

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Eukaryotic DNA-dependent RNA polymerases (Pols I–III) encode two distinct alpha-like heterodimers, with one being shared between Pol I and Pol III and the other unique to Pol II. These alpha-like subunits are evolutionarily conserved across all life forms and are proposed to be crucial for Pol assembly. These subunits are also involved in multiple stages of transcription, genome organization, and growth factor signaling. Studies on the alpha-like subunits have primarily focused on the Pol II subunits; however, the functional roles of the shared alpha-like subunits of Pol I and Pol III, POLR1C and POLR1D, remain largely unexplored. Notably, dysregulation of these shared subunits is linked to diseases such as cancer, developmental disorders, and neurological defects. Given the evolutionary conservation of the alpha-like subunits, we used AC19 and AC40, the yeast orthologs of POLR1D and POLR1C, to investigate the role of these subunits in Pol I and Pol III complex biogenesis and integrity. We utilized the Auxin-inducible degron (AID) system to trigger the proteasomal degradation of AC19 or AC40 and subsequently analyzed the impact of the loss of either subunit on the integrity of Pol I and Pol III complexes, as well as the protein abundance of other subunits within these complexes. Our findings reveal that the alpha-like subunits play distinct roles in the subunit expression dynamics and integrity of these polymerases. Our findings further suggest that Pol I and Pol III have differential requirements for the shared alpha-like subunits. These findings provide new insights into Pol I and Pol III subunit interactions and possibly points of regulation that could inform therapeutic strategies for diseases linked to Pol dysregulation.

112B

Investigating the mechanisms of replication-independent histone turnover in budding yeast

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An important aspect of chromatin dynamics is replication-independent histone turnover (H3 turnover), which refers to the exchange of the histone H3-H4 tetramer outside of S phase. This process has been mapped in multiple species, and high H3 turnover is primarily observed at promoters, enhancers, and the 3’ ends of genes. These turnover regions are also correlated with active transcription rates and RNA polymerase II density, suggesting turnover plays a role in gene activation, but the overall mechanism/function(s) of H3 turnover and its impact on transcription are poorly understood. We find that there are at least two categories of H3 turnover in budding yeast that differ in their genomic location and sensitivity to transcription: transcription-dependent turnover in gene bodies vs. transcription-independent turnover in gene promoters. Further, we find that positive supercoiling generated by transcription elongation can promote genic turnover, in line with its transcription dependence. Promoter turnover, on the other hand, occurs independently of transcription and many transcription factors/histone chaperones/remodelers; if anything, many of these factors suppress H3 turnover. However, a subset of promoter

turnover appears to be facilitated by the transcription repressor Tup1, revealing a potential role for H3 turnover in gene repression. This study overall aims to elucidate the specificity behind these two categories of H3 turnover, identify the processes/factors responsible, and determine more broadly how H3 turnover contributes to gene regulation.

113B

The contribution of aneuploid-associated RNA post-transcriptional modification to drug resistance

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Antimicrobial resistance has been identified by the World Health Organization as one of the top global public health and development threats, contributing to approximately 5 million deaths annually. Aneuploidy is a hallmark of emergent resistant cells undergoing antifungal treatment. The complete set of processes regulating the emergence of aneuploidy-associated resistance remains to be deciphered. Variations in post-transcriptional chemical modifications of RNAs have been suggested to contribute to drug resistance in cancer cells.

The most prevalent RNA modification is N^6-methyladenosine (m^6A). m^6A has been implicated in regulating various processes, including mRNA translation. Aneuploidy-associated copy number variation in genes encoding these proteins could lead to alterations in the cellular methylome. We hypothesize that aneuploid-associated RNA methylome variation contribute to antifungal drug resistance by regulating gene expression.

In this project, we aim to compare the RNA methylome of sensitive and resistant cells to the DNA-damaging agent, Zeocin, with the goal of identifying how methylation contributes to drug resistance. Here, I discuss preliminary data regarding strategies for generating aneuploid cells on demand through mitosis and meiosis, and selecting Zeocin-resistant cells with different ploidies.

These results set up the ground to explore the interplay between aneuploidy, RNA methylation, and drug resistance. Given that the number of antifungal drugs is limited while antimicrobial resistance is rising quickly, this project will allow to identify new drug targets to fight invasive fungal infections.

114A

Sequence features of a prion-like domain impact Ty1 retrotransposition in budding yeast

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The budding yeast *Saccharomyces cerevisiae* contains several families of retrotransposons which are prominent forces in genome evolution. The yeast Ty1 retrotransposon is a long-standing model for understanding the mechanisms used by retrotransposons and retroviruses. Ty1 replication requires the assembly of virus-like particles (VLP's) within the cytoplasm. The Ty1 Gag protein contains the structural capsid domain that assembles VLPs as well as an intrinsically disordered prion-like domain (PrLD). Both protein domains are essential for retrotransposition.

This projet investigates whether retrotransposition depends on sequence features of the Ty1 Gag PrLD, including domain length and amino acid composition. To test this we analyzed PrLD length and point mutants. Yeast transformation was followed by qualitative and quantitative retromobility assays to assess transposition frequency and Gag expression levels. Preliminary results suggest that alterations in PrLD sequence impact retrotransposition. These findings provide insight into how prion-like domains may alter retroelement activity.

115A

Gene Ontology Annotations: Bridging Experimental Data and Functional Knowledge

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The Gene Ontology (GO) knowledgebase is the world's largest source of information on the functions of genes. This knowledge is both human-readable and machine-readable, and is a foundation for computational analysis of large-scale molecular biology and genetics experiments in biomedical research. The GO resource encompasses multiple components: the ontology itself (a structured vocabulary), millions of annotations linking gene products to ontology terms, and computational tools for analysis.

The Gene Ontology is a controlled vocabulary developed to describe gene products in a species-independent manner. GO provides standardized terminology where precise, defined terms replace varied descriptions, enabling consistent communication about gene function across research communities. The Ontology is organized into three aspects: Molecular Function (MF; what a gene product does at the molecular level), Biological Process (BP; the larger biological programs accomplished by multiple or combined molecular activities), and Cellular Component (CC; where gene products are active). Each aspect is organized in a directed branching structure, allowing different levels of specificity as well as relationships between terms to be defined.

There are two types of GO annotations: standard GO annotations and GO-CAM Models. GO annotations link specific gene products to individual terms in the Gene Ontology, capturing what researchers have discovered and published into a standardized format. Each GO annotation includes at a minimum: a GO term, a gene product, a reference and an evidence code indicating the type of experimental or computational support. GO-CAMs (Gene Ontology Causal Activity Models) extend traditional annotations by combining multiple standard GO annotations into comprehensive

models that represent complete biological pathways. Understanding what experimental data supports annotations across all three GO aspects can help researchers design studies that facilitate more complete functional characterization.

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116A

Studying the interaction of Pyrin domain homologs and YopM using protein fragment complementation assay

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The innate immune system detects pathogens through multiple mechanisms, including pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Certain immune proteins function as sensors that monitor pathogen-induced disturbances. These proteins, such as pyrin, remain in an inactive state until they encounter pathogenic signals. Pyrin is a multidomain protein, one of which is the pyrin domain, a motif shared by numerous innate immune proteins. Approximately 27 human proteins contain pyrin domains. Yersinia pestis, the causative agent of plague, secretes six effector proteins into the host cytosol, one of which, YopM, was recently found to suppress the host immune response by interacting with pyrin, maintaining it in an inactive state and thereby inhibiting downstream immune signaling cascades. Yersinia pestis infects many different mammalian species, particularly rodents, despite their pyrin proteins being diverged from human pyrin (approximately 76.4% identity in the pyrin domain). It is not known what range of species' pyrin homologs can be bound by YopM. Pyrin is also polymorphic in the human population, and if any human variants block YopM binding, they could have been originally selected for during historical plague outbreaks. Finally, it is not known whether YopM interacts with pyrin domains from other immune proteins. In this study, we examine the effect of YopM on a library of 200 pyrin domain–containing homologs, both among other innate immune proteins and across different organisms, to determine whether YopM exerts similar inhibitory effects and whether innate immune suppression by YopM represents a conserved mechanism of action. We designed a library representing the pyrin domains of 27 human proteins, 174 species' pyrin homologs, and 42 SNP variants of human pyrin found in ClinVar. To single out the interactors, we will employ YopM as a bait protein in a protein fragment complementation assay (PCA). Therefore, after the screening using PCA, homologs that interact with YopM will be enriched and non-interactors will be eliminated; the enriched homologs will be identified by Illumina sequencing.

117A

Pomegranate Juice as a Longevity Mimetic: Insights into Metabolic Reprogramming and Mitochondrial Remodelling in *Saccharomyces cerevisiae* NCYC79

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Polyphenol-rich botanical extracts are known to modulate cellular and organismal health, yet the integrated genomic response to these complex mixtures remains partially characterized. In this study, we performed a comprehensive RNA-seq analysis of *Saccharomyces cerevisiae* NCYC79 treated with 10% Pomegranate juice (PJ) to elucidate its impact on growth, metabolism, and longevity. Our results identified 1,703 differentially expressed genes (1,002 up-regulated; 701 down-regulated), revealing a robust transition from a proliferative state to a survival-oriented "quiescent" profile. Key findings include a significant upregulation of the GABA shunt (*UGA1*, *UGA2*) and the TCA cycle (*IDH1*, *IDH2*, *CIT1*), suggesting a metabolic shift toward high-efficiency respiration. This was accompanied by a marked induction of PNC1, a master regulator of nicotinamide clearance and Sirtuin-mediated longevity, and the downregulation of pro-aging factors such as TMA23 and FOB1. Furthermore, we observed a unique modulation of mitochondrial dynamics: while oxidative phosphorylation subunits (*COX4*, *ATP2*) were significantly upregulated, the mitochondrial-ER tethering complex (ERMES) subunit *MDM34* was suppressed, and the fission recruiter *FIS1* was induced. This suggests a "damage containment" strategy characterized by increased respiratory capacity and mitochondrial network fragmentation. Finally, the sharp downregulation of ribosome biogenesis and mRNA splicing machinery mimics the effects of TOR pathway inhibition. Taken together, these data suggest that pomegranate extract serves as a potent longevity mimetic by coordinating metabolic reprogramming with organelle quality control, providing a framework for exploring the therapeutic potential of pomegranate-derived compounds in aging and metabolic disorders.

118A

Wild yeast deletion collections and the genetic background effect on genetic networks

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An individual's genome, their genetic background, contains a highly similar set of ~6000 genes but encompasses a distinct set of variants. By leveraging the genetic and phenotypic diversity of *S. cerevisiae*, we can begin to systematically assess the influence of different genetic backgrounds on various phenotypes, including complex genetic networks. To do so, we generated a universal CRISPR-Cas9 plasmid library with gRNAs designed to target and delete all yeast genes in any *S. cerevisiae* strain. Using this system, we constructed seven genome-wide deletion collections in a set of diverse *S. cerevisiae* genetic backgrounds. By comparing viability phenotypes in wild yeast to those of the S288c reference lab strain, we identified mutants with altered essentiality dependent on genetic background and mapped several modifiers of these conditionally essential genes. The mutant strain collections are compatible with Synthetic Genetic Array (SGA) analysis, allowing us to characterize the extent to which genetic background modulates the global yeast genetic interaction network. As a proof-of-concept, SGA analysis was performed on an array of ~300 non-essential deletion mutants in five yeast backgrounds. We find that genetic interactions are more prevalent in natural backgrounds compared to the S288c laboratory reference strain, yet they remain functionally coherent. Systematic genetic interaction analysis has the potential to identify new functional connections that may go unnoticed when

limiting screens to the reference S288C genetic background. In particular, SGA screening in multiple backgrounds enables further characterization of the remaining ~500 genes of uncharacterized function within the S288C genome.

Keywords: Genetic interactions, Gene essentiality, Conditional essentiality, Genetic background, Genetic network

119A

Systematic comparison of transcription factor binding locations and perturbation responses

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Determining the targets of each yeast transcription factor (TF) is a longstanding goal. To that end, there have been multiple attempts to perturb each TF and measure the effect on gene expression (perturbation response) and to measure the genomic binding locations of each TF (binding location). First produced the mid 2000s, such datasets continue to be produced today. Perturbation methods include gene deletion and overexpression and TF degradation. Location assays include ChIP-chip, ChIP-exo, ChEC-seq, and Transposon Calling Cards (our data, reported here for the first time). However, finding, harmonizing, and comparing these datasets has been challenging, in part because they were processed using different statistical approaches, significance thresholds, and promoter definitions. We have created a single repository for all datasets and a website (https://tfbindingandperturbation.com) and application programmer interface through which they can be selected, compared, and analyzed. Annotated metadata include growth conditions, time points, and experimental methods. Researchers interested in particular TFs or genes can synthesize information from many datasets on our site while systems biologists can select and download full datasets, common TFs across datasets, specific growth conditions, and so on. Visitors can choose between published analyses and uniformly reprocessed data. Currently, the repository houses 11 datasets totaling 1,918 binding profiles on 851 different proteins and 3,571 PR profiles on 1,514 different proteins.

We systematically compared all perturbation response and binding location datasets for the first time. We found that the median number of responsive genes among the 25 genes whose promoters are most strongly bound by a TF is 4, spotlighting a persistent scientific mystery. Across TFs, the 2004 ChIP-chip data and 2007 TFKO data had the lowest response rates. Our Calling Cards data tended to have the most responsive genes among the 25 most strongly bound (overall median 7), followed by ChEC-seq and then ChIP-exo. We will present results of analyzing and comparing these datasets and demonstrate the analysis tools available through our website. Insights include the effects of different promoter definitions, experimental techniques, and analysis methods on the overlap between bound and responsive promoters. This resource provides a new opportunity for integrated, cross-dataset analyses at the levels of individual biological processes and of the complete gene regulation system.

120A

Genetic mapping identifies loci that affect survival during antifungal treatment in *S. cerevisiae*

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Fungal pathogens, and the accelerating rise of antifungal resistance, are a continuing public health threat. Although new antifungal compound classes are progressing through clinical trials, resistance to both established and emerging therapeutics remains a critical problem. Given that many antifungal drug targets and their associated molecular pathways are conserved between *Saccharomyces cerevisiae* and pathogenic fungi, natural genetic variation in *S. cerevisiae* offers a powerful system for uncovering mechanisms of antifungal survival. We hypothesized that standing genetic variation could reveal known and previously uncharacterized determinants of resistance, tolerance, and persistence. To test this, we adapted a bulk segregant mapping approach for use in microtiter plates, substantially increasing throughput relative to previous methods. In preliminary experiments with fluconazole, we identified a genetic locus containing its known target *ERG11*, as well as additional loci associated with survival. These novel loci contain genes not previously implicated in antifungal resistance. Together, these findings demonstrate that this high-throughput mapping strategy could expand our understanding of fungal survival mechanisms. Future work will more broadly assess the effects of extant variation on additional antifungal compounds and define how the genetic variation in mapped loci leads to antifungal survival.

121A

Sensitivity analysis of GRNmap and new features for GRNsight: open source software for dynamical systems modeling and visualization of small-scale gene regulatory networks in yeast

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A gene regulatory network (GRN) is a set of transcription factors that regulate the expression of genes encoding other transcription factors. The dynamics of GRNs explain how gene expression changes over time. GRNmap is an open-source MATLAB package that uses ordinary differential equations to model dynamics of small-scale GRNs. The program estimates production rates, expression thresholds, and regulatory weights for each transcription factor in the network based on experimentally derived gene expression and degradation rate data and then performs forward simulations of model dynamics. While the model has been successfully used to understand networks of 15-20 genes that regulate the response to the environmental stress of cold shock in budding yeast, *Saccharomyces cerevisiae*, we wanted to closely examine how it works on a smaller scale to determine parameter sensitivity. All 21 possible “toy” networks of 3 nodes and 4 edges were created, which fall into 11 families of network motifs such as “feed-forward loops”, “mutual-in”, and “fan-in”, among others. Parameters were estimated from simulated expression data output when known arbitrary weight parameters (-2, -1, 1, 2) underwent a forward simulation. Then the simulated data was used to estimate the parameters again. Comparison of the known to estimated parameters showed

that estimating production rates in addition to weights and thresholds reduced the accuracy of the results. The model was also sensitive to the direction and magnitude of the arbitrary weight parameters for networks with the same connectivity. To better understand the influence of the weight parameters, we generated all twenty-four possible weight permutations for a few network motifs. By evaluating the permuted network model results, we can better understand why certain network motifs are more prevalent in natural GRNs. To facilitate interpretation of model results, we developed GRNsight, an open-source web application for visualizing models of GRNs, automatically displaying a graph where edges are color-coded based on the activation and repression relationships, and nodes are color-coded with time course gene expression data. Backend yeast gene expression, gene regulatory network, and protein-protein physical interaction databases based on data from NCBI GEO and SGD AllianceMine allow users to generate a network, color the nodes with expression data, and export an Excel workbook for modeling in GRNmap. Software is available at http://kdahlquist.github.io/GRNmap/ and https://dondi.github.io/GRNsight/.

122A

The *Saccharomyces cerevisiae* pan genome: an approach

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In the past few years it has become much easier to generate telomere to telomere complete fungal genomes. Our goal, taking advantage of this, is to create a pan genome application based on a different approach than has been typically used for fungal genomes, based on defining an element based on conserved location and orientation relative to the flanking elements, not based strictly on sequence conservation. These genomic elements include genes encoding proteins and RNA products, transposable elements, centromeres, telomeres, pseudogenes, gene remnants, elements from the mitochondrial and plasmid genomes, and identifiers such as the central region of the genome and the sub-telomeric region. This use of flanking elements is why the approach works best on complete genome sequences. These elements include introgressed copies, and genes with stop codons/frame shifts/deletions/TY insertions that we consider putative null alleles, as alleles not pseudogenes as long as they are at the same location and orientation. Pseudogenes are at atypical locations for the species and related species. The goal of this pan genome project is to format the data to allow for a wide diversity of queries across this “genome sequence of a species”.

The approach we are developing allows for not only queries within the pan genome of a single species, but eventually between the pan genomes of related species. Comparison of the genomes of *S. cerevisiae* and *Ashbya gossypii* has shown us that gene order conservation is in general a more powerful method of identifying orthologs than sequence conservation, thus is the basis of our pan genome approach. “What are the differences in the core set of elements between species 1 and species 2?” Our starting point in this work is the 93 *S. cerevisiae* genomes we have worked on where we are currently completing the telomere-to-telomere sequence and also the thousands of other *S. cerevisiae* complete or near complete genomes that others have generated. Initial work on this has allowed us to identify TY7 in *S. cerevisiae*, gene remnants, variation in the set of tRNA genes, the set of core and variable elements, sites of translocation, variation in tandemly amplified genes, and the boundary of the sub-telomeric region where the genome organization shifts from highly conserved element order to more variable. Our initial arbitrary definition of core elements is elements found at the same location and orientation in 95% of strains.

123B

Functional Synergy Partially Explains Why Most Transcription Factor Binding is Non-functional.

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A simple conception of transcriptional regulation is that transcription factors (TFs) regulate the genes in whose promoters they bind. However, it has been shown repeatedly that most TF binding in promoters is non-functional, in the sense that removing the TF does not measurably affect the expression of the gene. To determine whether this might be an artifact of chromatin immunoprecipitation (ChIP), we created a comprehensive TF binding location dataset using Transposon Calling Cards, which works by linking a TF to a transposase, recovering inserted transposons with flanking DNA, and sequencing. More of the genes whose promoters a TF binds respond to perturbations of that TF in our data, compared to ChIP data. Nonetheless, most of the strongly bound promoters still do not respond to TF perturbations.

Next, we hypothesized that a gene’s response to perturbation of a TF bound in its promoter can depend on which other TFs are bound there, a phenomenon we call *functional synergy*. Functional synergy is distinct from cooperative binding, which explains where TFs bind, not how they affect transcription once they are bound. To evaluate this potential explanation, we fit mathematical models for predicting responses to TF perturbations from the measured binding locations of all TFs, not just the perturbed TF (pTF). The models include TF-TF interaction terms representing functional synergy. After rigorous statistical testing, we found 38 highly significant interactions between the binding signals of pTFs and those of other TFs, which we call modifier TFs (mTFs). Examples include Dal80-Gzf3, Cha4-Lys14, Msn2-Gal4, and Mig1-Cbf1. These interactions are independent of any effects the mTF may have on the pTF’s binding locations, as our models directly incorporate the measured binding locations of both factors. The presence or absence of modifier TFs helps explain why genes whose promoters are similarly bound by a TF respond differently to perturbations of that TF.

This work sets the stage for exciting future research. One direction is to investigate the mechanisms of functional synergy. For example, pTF and mTF might regulate different phases of transcription. Alternatively, they might cooperatively recruit a third factor whose binding locations were not measured. Another direction is to test the model’s ability to predict perturbation responses in different growth conditions, given binding location data from those conditions. The ability to predict perturbation responses in new conditions would be a significant advance in regulatory systems biology.

124B

Metabolic Regulation of TORC1 During Amino Acid Starvation

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Cells sense amino acid levels and either import amino acids or upregulate biosynthetic pathways. When starved of amino acids, TORC1 activity decreases leading to autophagy, reduced translation, and arrest at G1. Glyphosate-based herbicides (GBH) such as RoundUp target Aro1 in the shikimate pathway (aromatic amino acid biosynthetic pathway) and inhibit TORC1. There is genetic variation between yeast strains and their response to GBH. Loss of Aro1

is lethal in glyphosate-resistant strains and cannot be rescued with aromatic amino supplementation. The EGO complex regulates TORC1 activity and assists in the reactivation of TORC1 after starvation. When the EGO3 allele from a resistant strain is replaced with EGO3S288cfrom the sensitive strain, it conferred increased resistance to GBH and rapamycin in the GBH resistant strain. Ptr3, a component of the amino acid-sensing complex SPS, was also essential in the GBH-resistant strain due to synthetic lethality with EGO3. While the presence of PTR3 from the resistant strain increased viability during starvation, the presence of the PTR3 from the sensitive strain prevented return to the cell cycle. Ptr3 oligomerization is an important step in signaling, and polymorphism affects the compactness of the oligomers but not interactions with other members of the SPS complex. Thus, increased SPS signaling increased TORC1 activity reducing survival during starvation. During starvation tryptophan is converted to auxin (indole-3-acetic acid (IAA)) which directly inhibits TORC1. Proper regulation of TORC1 is essential for long term survival during starvation. When cells are unable to synthesize auxin, they must rely on EGO for TORC inhibition.

125B

Uncovering the Basis of Killer Toxin Resistance in *Saccharomyces cerevisiae*

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Killer toxins are a diverse group of antifungal proteins secreted by fungi to gain advantages over competitors. Many killer toxins are encoded by dsRNA and dsDNA viruses and virus-like elements in fungi, share little or no sequence similarity, and have wide-ranging mechanisms of action. These mechanisms of action include pore-formation (K1 and K2 toxins) and tRNA and rRNA cleavage (zymocin-like toxins). Recently, an uncharacterized defense factor, Ktd1, against a killer toxin, K28, was discovered in *Saccharomcyes cerevisiae*. *Ktd1* belongs to one of the largest and most variable groups of genes in the *S. cerevisiae* population, the *DUP240* family, which is composed of mostly uncharacterized genes. Knowing this, we hypothesized that the *DUP240* genes may be a killer toxin defense family. Using a diverse panel of *S. cerevisiae* strain crosses subjected to K28 variants and other killer toxins, we are performing Bulk Segregant Analysis, which allows for finite mapping of regions of the genome associated with killer toxin resistance and the effect to which they contribute to killer toxin resistance in the *S. cerevisiae* population.

126B

A global map of functional module crosstalk in the yeast genetic interaction network

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Genetic interaction networks provide a powerful framework for mapping functional relationships between genes, yet gene-centric analyses often obscure higher-order organization among biological processes. Using curated functional modules—including cellular components, pathways, and protein complexes—we analyzed the global *Saccharomyces cerevisiae* genetic interaction dataset derived from Synthetic Genetic Array (SGA). We developed a permutation-based enrichment test and systematically tested negative and positive genetic interaction crosstalk between modules and between genes and modules, producing enrichment profiles for genes and modules. We used these profiles to build a module similarity network that maps relationships among biological functions. This analysis revealed a global map of functional module crosstalk. Distinct biological processes emerged as coherent subnetworks, including a densely connected metabolic module network that is weakly resolved in the gene-level genetic interaction similarity network from the same dataset. Module-level analysis reveals systems-level relationships that are hard to detect from gene-level analyses.

127B

Elucidating the molecular grammar of a retrotransposon prion-like domain in budding yeast

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Retrotransposons and retroviruses shape genome evolution and can negatively impact genome function. The Ty1 retrotransposon of the budding yeast *Saccharomyces cerevisiae* provides an excellent model for studying fundamental mechanisms of retroelement propagation. Retrotransposition of Ty1 requires a prion-like domain (PrLD) within the Gag protein that contains similar amino acid composition to known yeast prions. Here, we probe the sequence constraints governing Ty1 PrLD function by investigating various length, charge, and amino acid point mutations. We define length and charge limitations for Ty1 retromobility and find dispensable and critical amino acids in the PrLD sequence. Future work will continue characterizing the sequence parameters that dictate the rules of the Ty1 PrLD's "molecular grammar".

128B

The effects of genetic variation on metal resistance and differential sorption distribution in yeast.

Luis S Martinez, Jennifer S Gallagher Biology, West Virginia University

Although government action has reduced the prevalence of lead (Pb) pollution and poisoning around the world, exposure to any amount of Pb continues to be a problem, especially in poor and rural communities. *Saccharomyces cerevisiae*'s ability to rapidly reproduce and mutate in lab environments represents a valuable opportunity to explore genetic strategies for metal remediation along with a demonstrated ability to bind and bioaccumulate heavy metals in numerous studies. The exact genetic and mechanical means by which yeast cells can accumulate Pb have not been thoroughly explored. In our project we have used different yeast strains to investigate this gap in knowledge. Through in-lab evolutions we have evolved a mutant strain of yeast that has a higher resistance to Pb. Through a novel method, we have observed that there is a significant difference between the extracellular adsorption and internal absorption of Pb. We measured this by exposing equal numbers of yeast to metals and washing them with either ultrapure water or a chelating solution, Ethylenediaminetetraacetic acid (EDTA). We measured accumulated metals with ICP-OES and our preliminary data suggests that evolved strain RM11.3 has a higher resistance to Pb in spite of absorbing a higher proportion of accumulated Pb. We have sequenced the evolved strain and are in the process of investigating genetic mutations that might be responsible for these observations. Furthermore, we are working on validating our novel method to determine the veracity of differential lead accumulation in yeast. We propose that our research will give a greater insight into the mechanisms of lead accumulation in yeast and be a steppingstone for further research and applications with positive implications for human and environmental health.

129B

Development of a high-throughput genome-wide method to assess Ty1 retrotransposon insertion upstream of tRNA genes in *Saccharomyces cerevisiae*

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Transposable elements are mobile DNA elements found in nearly all eukaryotes. Retrotransposons, a sub-class of transposable elements, mobilize via RNA intermediates. In the genome of the S288C reference strain of *Saccharomyces cerevisiae* , Ty1 is the most abundant retrotransposon, with ~38 copies per genome. Because the structure and life cycle of retrotransposons resemble those of retroviruses, studying Ty1 in yeast can provide insights into related human retrotransposons and retroviruses. After reverse-transcription of Ty1 mRNA into complementary DNA (cDNA), Ty1 cDNA integrates upstream of genes that are transcribed by RNA Polymerase (Pol) III, such as transfer RNA (tRNA) genes. Ty1 cDNA integration into the genome is mediated by Ty1-Integrase and physical interaction with RNA Pol III. However, other host factors are likely involved because Ty1 can insert up to 1 kilobase upstream of tRNA genes, far beyond the RNA Pol III footprint, and insertion coincides with nucleosome positioning. Our aim was to develop and validate a high-throughput method to assess Ty1 insertion upstream of tRNA genes and identify host factors required for Ty1 integration. Because non-essential genes have already been queried for Ty1 mobility, we focussed on the essential temperature sensitive (ts) allele collection. To develop our approach, S288C was transformed with a Ty1-donor plasmid containing a galactose-inducible Ty1 element with a synthetic barcode sequence (SSB) tag within the long terminal repeat of the Ty1 element. After galactose induction of the Ty1 element, we identified Ty1 insertion sites at 10 select tRNA genes using a multiplex polymerase chain reaction (PCR) with primers that recognize sequences immediately upstream of the tRNA genes and a primer for the SSB tag. Amplicons derived from the multiplex PCR reactions were sequenced using Illumina technology to identify their insertion sites. We mapped Ty1 insertion patterns and frequencies upstream of the 10 tRNA loci and identified differences in Ty1 insertion profiles between wildtype and ts mutant strains with defects in DNA replication, chromosome division, RNA Pol III machinery and chromatin remodelling. We confirmed the utility of our method for high-throughput testing of Ty1 targeting in ts mutant strains. All categories of ts mutants had defects in Ty1 insertion with respect to the number of insertion sites, frequency of insertion and the profile of tRNA genes that were targeted.

130B

Sequence constraints on the Ty1 retrotransposon in the budding yeast *Saccharomyces cerevisiae*

Mackenzie Streeter, Alexa MacKersie, Awesome Abraham, Samantha Wee, Sean Beckwith Hope College

Retrotransposons and retroviruses are key elements in genome evolution. The Ty1 retrotransposon of the budding yeast Saccharomyces cerevisiae provides a useful model for the study of fundamental mechanisms of retroelement life cycles. Ty1 retrotransposition requires an intrinsically disordered prion-like domain (PrLD) within the Gag protein that contains amino acid sequence composition similar to other known yeast prions. By tagging Ty1 with an auxotrophy marker we can measure retrotransposition. We are investigating the sequence constraints including length and charge by testing a series of mutant PrLDs. Our work underscores the importance of sequence composition in Ty1 PrLD function and highlights a gap in our understanding of the molecular grammar of intrinsically disordered domains.

131B

High-throughput Screening of Putative Anti-CRISPR Proteins against SpyCas9 and SauCas9 in Yeast

Dinie Zheng, Emma Fidacaro, Michael Chambers, Meru Sadhu NHGRI/NIH

Bacteria and bacteriophages are engaged in a coevolutionary arms race and therefore, have evolved a number of mechanisms to fight or defend against one another. One of the defense mechanisms that bacteria have evolved are the CRISPR-Cas systems, and bacteriophage have thus evolved anti-CRISPR (Acr) proteins to inhibit CRISPR-Cas systems. Currently, about 100 Acrs have been discovered, 38 of which specifically target type II-A CRISPR-Cas systems. We aim to expand the breadth of known Acrs in two directions. First, we are studying homologs of known type II-A CRISPR-Cas system-inhibiting Acrs, to determine the extent of conservation of Acr function. Second, we are characterizing potential de novo Acrs to determine the extent of additional Acr proteins. Over 2500 putative type II-A genes were synthesized to be screened. We developed a high-throughput screening method to screen the ability of the Acrs to inhibit eukaryotic CRISPR editing by the Cas9s from *Streptococcus pyogenes* (SpyCas9) and *Staphylococcus aureus* (SauCas9) in the yeast *Saccharomyces cerevisiae*.

Our high-throughput screens have found over 250 Acrs that inhibit SpyCas9 and over 25 Acrs that inhibit SauCas9. These Acrs include diverse homologs of AcrIIA2, AcrIIA4, AcrIIA5, etc., with some sharing as little as 40% protein sequence identity to the canonical proteins. We have also revealed two potential de novo Acrs that inhibit SpyCas9. Further work will be focused on characterizing the positive hits from the screens and screening more putative Acrs against these type II-A Cas9s. Thus far, we have found that very few de novo predicted Acrs inhibited SpyCas9, which could suggest that the space of Acrs useful for inhibition of SpyCas9 in eukaryotic biotechnological settings has been largely mapped. This research will contribute towards the understanding of Acr diversity and expand upon the arsenal of Acr genes available for eukaryotic biotechnological applications.

132A

Single Cell Analysis of Rejection of Homologous Recombination Intermediates during Single Strand Annealing

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Double-strand breaks (DSBs) threaten genomic integrity and, if left unrepaired or repaired with low fidelity, can cause chromosomal rearrangements and deletions. Cells in all domains of life have developed high fidelity repair mechanisms. For example, heteroduplex rejection serves as an anti-recombination mechanism to prevent DSBs from utilizing a divergent donor template for repair. Heteroduplex rejection can be initiated by MSH proteins, which recognize DNA mismatches in heteroduplex recombination intermediates and recruit helicase factors to unwind them. Little is known about the localization and timing of heteroduplex rejection within a cell. We utilized in baker's yeast a homologous recombination repair pathway, single-strand annealing, that repairs DSBs between repeat sequences (Sugawara, PNAS 101:9315). Divergent donor sequences generate a mismatched heteroduplex intermediate, which can be recognized and undergo heteroduplex rejection. In this system, which specifically requires Rad52 functions, a DSB is induced between homologous or

divergent repeats, and fluorescently tagged Rad52 is used to visualize repair intermediates. Using live cell fluorescence microscopy, we found that Rad52 foci persist significantly longer in strains undergoing heteroduplex rejection. This behavior is lost upon deletion of Msh6, which disrupts mismatch recognition and prevents heteroduplex rejection. We found that Rad52 foci in strains undergoing heteroduplex rejection show enhanced sub-diffusive motion compared to the behavior of unbroken chromosomal loci. DSBs that cannot be repaired and those repaired using homologous repeats display diffusion dynamics indistinguishable from unbroken chromosomal loci, indicating that enhanced sub-diffusion is specific to heteroduplex rejection. This heteroduplex rejection diffusion behavior depends on Tub3, a component essential for the formation of DNA damage–induced microtubules, which have been implicated in the relocation of poorly repaired DSBs to the nuclear periphery (Oshidari, Nat. Comm. 11, 695). DNA undergoing heteroduplex rejection eventually relocates to the nuclear periphery, but relocation does not appear to influence anti-recombination between divergent sequences. These results reveal that heteroduplex rejection enforces a distinct state characterized by extended Rad52 persistence and enhanced sub-diffusive motion, linking anti-recombination to regulated chromosomal mobility.

133A

Defining and mapping the mutagenic effects of the cytidine deaminase APOBEC3C Shamitha Aravind[1,2], Grant W Brown[1,2] [1]Department of Biochemistry, University of Toronto, [2]Terrence Donnelly Centre for Cellular and Biomolecular Research

Mutation signatures are characteristic patterns of mutagenesis that reflect the underlying processes that shape a genome. The seven cytidine deaminases that comprise the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3) family can deaminate genomic DNA and cause unique mutation signatures. Despite being expressed at relatively higher levels among the *APOBEC3* family across a broad range of cell and tissue types, the mutational consequences of APOBEC3C (A3C) activity in the human genome are poorly defined. While A3C largely shares the 5'-TCW trinucleotide preference of A3A and A3B, A3C has specific nucleotide preferences at the -2 position to the deaminated cytidine which relax this trinucleotide sequence preference. Analysis of whole-genome sequencing data from multiple cancer types shows the presence of mutational patterns with high similarity to those inflicted by A3C. My approach will identify the consequences of A3C mutagenesis in human genomes.

134A

Investigating the origin and nature of half-crossover cascades in *Saccharomyces cerevisiae* Juan Lucas Argueso, Ruth A Watson, Via M Lawson Environmental and Radiological Health Sciences, Colorado State University

A half-crossover cascade (HCC) is a mutagenic sub-pathway of homologous recombination (HR), where a single DNA double-stranded break (DSB) is able to trigger the formation of not just one, but many chromosomal rearrangements. An asymmetrical HR repair structure can be formed when only one of the two broken ends of a DSB is resected, engages and invades an intact homologous donor sequence. At this point, repair of the broken recipient sequence may proceed through break-induced replication (BIR), extending the broken 3' end through the telomere of the donor chromosome. Alternatively, the single-end invasion HR intermediate may be processed by structure selective endonucleases (SSEs) to form a half-crossover, in which the initial single-ended DSB is healed through a translocation, while at the same time breaking the donor molecule, and worse, leaving it with only one DNA end to engage in another futile round half HR repair. This may spawn yet another half-crossover rearrangement, resulting in the cascading nature of this mechanism. Thus, HCCs can lead to cycles of rearrangements, until one round of BIR occurs and finally ends the mutagenic series. We have developed an experimental system in *S. cerevisiae* haploids to select for, quantify, and genomically characterize clones that carry HCC rearrangements. The process begins through the induction of a CRISPR-Cas9 DSB near the end of chromosome VI (Chr6), with a repetitive sequence on the centromeric side, but no homology on the telomeric side. The non-essential telomeric side is lost, and only the centromeric side of the DSB is able to engage in ectopic half HR repair. This eventually triggers HR between ectopic homeologous repeats inserted on Chr10 and Chr9 (99.5% nucleotide sequence identity), which leads to the formation prototrophic cells that can be selected for in dropout media. Subsequent phenotypic analysis of auxiliary markers is used to infer the nature of the rearrangements present, which are then validated through pulse-field karyotyping and long-read sequencing. We have extensively characterized this system, including identifying multiple clones that show the unambiguous signature of HCC: Chr10-Chr9 translocations with repeat sequences at their junction that contain SNPs derived from initially broken Chr6. We will describe this system, as well as an initial characterization of its genetic dependencies, including the role of BIR (*pol32Δ*), SSEs (*rad1Δ*, *yen1Δ* and *mus81Δ*), and mismatch repair (*msh6Δ*).

135A

Live Cell Imaging of Meiotic Homolog Pairing in Polyploid Yeast David Bai, Tadasu Nozaki Biology, University of Massachusetts Amherst

Diploid (2n) organisms contain two sets of homologous chromosomes, each of which must spatially pair during meiosis for crossover formation. In polyploid organisms (>2n), pairing becomes more complicated, as 3 or more homologous chromosomes must each pair during meiosis. The question of how these chromosomes pair and how polyploidy affects the progression of meiosis in vivo remains unsolved. To observe pairing in polyploid cells, budding yeast containing tagged fluorescent loci on chromosome II were prepared. Triploid (3n) and tetraploid (4n) yeast strains were constructed by disrupting the MATa or MATα locus and repeated mating. Ploidy level was then confirmed by flow cytometry and number of fluorescent spots. After meiotic induction, long timelapse live-cell imaging of the polyploids allowed for observation of chromosomal pairing during meiotic prophase I. The resulting imaging data showed that the pairing of chromosomes is very stable throughout meiotic prophase. Also, bivalent pairing occurs in the majority of cases, with trivalent and quadrivalent pairing being less frequent. This suggests that chromosomes, once paired, are protected from further pairing, even if there are still unpaired chromosomes in the nucleus. How trivalent and quadrivalent pairing during meiotic prophase affects the propagation of the synaptonemal complex and crossover interference in polyploids remains an open question.

136A

Global licensing of meiotic recombination by a threshold mechanism

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Sexual reproduction relies on meiosis, a specialized cell division that halves the chromosome number in diploid progenitor cells to produce haploid gametes. During meiotic prophase, homologous chromosomes must align, synapse and form crossovers to ensure their proper segregation at the first meiotic division. Hop1 is a highly conserved HORMA-domain protein and master regulator of meiotic prophase events. These include the initiation of recombination by DNA double-strand break (DSB) formation, inter-homolog template bias, and meiotic checkpoint signaling.

Our work suggests that a threshold level of nuclear Hop1 is critical for successful meiosis. We identified a mutant allele of *HOP1* that is defective for nuclear import due to two lysine-to-arginine substitutions in predicted nuclear-localization signals (NLSs). *hop1-2KR* produces <1% viable spores and almost no DSBs, analogous to a *hop1*Δ null mutant. Given that the hop1-2KR mutation abolishes DSB function, we were surprised to discover that chromosomal foci and ChIP signals of hop1-2KR were ~40% of control levels and their distribution was normal. The possibility that hop1-2KR is dysfunctional for both nuclear localization and DSB formation was ruled out because fusion to a strong NLS (from SV40) restored nuclear localization, DSB formation, and spore viability.

These observations suggest that a threshold of nuclear Hop1 may be required to trigger DSB formation globally. Consistently, increasing hop1-2KR levels with a titratable promoter (copper-inducible, P_{CUP1}) produces a remarkable all-or-nothing phenotype in which meiosis either succeeds, producing four viable spores; or fails, producing only dead spores. Absent are tetrads with two viable spores, diagnostic of partially defective meiosis in which one or a few chromosomes mis-segregate. Moreover, titrating hop1-2KR levels progressively increased viability from zero to 90%, while maintaining the all-or-nothing spore viability pattern.

A threshold model of global DSB licensing by Hop1 is being explored by correlating spore viability with hop1-2KR levels and DSB formation in individual cells. How could a threshold mechanism of global DSB licensing work? Blitzblau and Hochwagen showed that ongoing replication globally inhibits DSB formation via DNA-damage response and cell-cycle kinases. We are testing whether this anti-DSB checkpoint is active when levels of nuclear Hop1 are low.

137A

The evolution of genome instability in wild and clinical isolates of Baker's Yeast

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The tendency of a cell to undergo changes in its genome is known as genome instability. While it is commonly thought that high genome instability is deleterious to cells, genome instability is a hallmark of cancer progression and fungal pathogens resistant to treatment. Our current understanding of genome instability, though, is solely from laboratory isolates that are very genetically stable, which is likely not representative of what we observe in the wild. This emphasizes the importance of studying the frequency at which genome instability rises in different yeast backgrounds, specifically in the absence of selection for such genetic changes. For example, a strain with a higher genome instability would have a higher chance of mutating a particular gene more often than a strain with a lower frequency of genome instability, which could confer a fitness advantage. Using natural isolates of *Saccharomyces cerevisiae*, we generated a collection of strains that are heterozygous at the *URA3* locus (*URA3*/*ura3*Δ). This strategy allows us to select for events in which cells have lost the *URA3* gene and thus experienced genome instability. With these strains, we performed a mutation accumulation evolution experiment in rich media conditions and found that genetic background alters genome instability over time. Our work shows that unlike laboratory isolates, clinical isolates that we tested showed significantly higher frequencies of genome instability. Furthermore, we observe that clinical yeast strains with higher genome instability also exhibit higher variance in colony morphology.

138A

Yeast ORFan interactions with RNA-processing protein Npl3 and telomere maintenance

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The budding yeast RNA-processing protein, Npl3, is involved in the maintenance of telomeres by regulating the expression of non-coding telomeric transcripts (TERRA) and slowing the rate of senescence in telomerase mutants. Without telomerase, progressively shortening of telomeres with rounds of replication eventually leads to cell senescence caused by cell cycle arrest. The exact mechanism of regulation by Npl3 remains elusive because of its versatile roles in the cell, especially during gene expression. Npl3 genetically and physically interacts with 1200+ genes and gene products, many with unknown functions. With the NSF-funded Yeast ORFan project, we decided to choose GUFs (genes with unknown function) that also interacted with NPL3 as a way to explore their functions and see how Npl3 may work to maintain telomeres. We examined MAG2 which encoded a RING-finger type E3 ubiquitin ligase and WWM1 which encodes a WW domain-containing protein, based on bioinformatics analyses. While neither deletions affected the rate of senescence in telomerase mutants indicating that they are not required for telomere maintenance, they did cause accelerated senescence of tlc1 npl3 mutants. These results suggest that Mag2 and Wwm1 work separately and downstream of Npl3's role at the telomeres. We also examined the GUF YOR008C-A which has genetic interactions with NPL3, and yor008c-a mutants have slightly long telomeres from genome-wide screens. However, we did not observe any differences in the rate of senescence between tlc1 single and tlc1 yor008c-a double mutants; there were no additional changes when YOR008C-A was deleted in tlc1 npl3 mutants. Thus, the YOR008C-A gene product likely does not play a role in telomere maintenance or work in the same pathway as Npl3. We plan to update our bioinformatics analyses and devise additional experimental plans to characterize these and other GUFs while continuing our efforts to describe the role of Npl3 at telomeres.

139A

Evaluating genome instability caused by cancer-associated mutations in the mismatch repair gene *MSH3*

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The cBioPortal for Cancer Genomics is a rich community resource that combines cancer genomics data with clinical profiles of tumor samples. We found that 10% of patients in the database with Uterine Corpus Endometrial Carcinoma have mutations in *MSH3*. *MSH3* encodes a subunit of MutSb, which recognizes DNA lesions as the first step of the highly conserved DNA mismatch repair pathway. Because many of these mutations are characterized as variants of uncertain clinical significance, we sought to investigate the equivalent mutations using budding yeast *S. cerevisiae* as a model system. In addition to an ATPase-deficient strain (*msh3-K797A*), we constructed five yeast strains encoding Myc-tagged versions of mutant Msh3 protein. We will evaluate the effect of these mutations on microsatellite repeat instability, DNA repair using the direct duplication recombination assay, and protein abundance via western blot. These studies will shed light on how cancer-associated mutations in *MSH3* may influence genome instability.

140B

Investigating the role of Rad1 in facilitating the initiation half-crossover cascades in *Saccharomyces cerevisiae*

Via M Lawson, Ruth A Watson, Juan Lucas Argueso Environmental and Radiological Health, Colorado State University

A half-crossover cascade (HCC) is a mutagenic sub-pathway of homologous recombination (HR), where a single DNA double-stranded break (DSB) is able to trigger the formation of not just one, but many chromosomal rearrangements. An asymmetrical HR repair structure can be formed when only one of the two broken ends of a DSB is resected, engages and invades an intact homologous donor sequence. At this point, repair of the broken recipient sequence may proceed through break-induced replication (BIR), extending the broken 3' end through the telomere of the donor chromosome. Alternatively, the single-end invasion HR intermediate may be processed by structure selective endonucleases (SSEs) to form a half-crossover, in which the initial single-ended DSB is healed through a translocation, while at the same time breaking the donor molecule, and worse, leaving it with only one DNA end to engage in another futile round half HR repair. This may spawn yet another half-crossover rearrangement, resulting in the cascading nature of this mechanism. Thus, HCCs can lead to cycles of rearrangements, until one round of BIR occurs and finally ends the mutagenic series. In preceding work (*see the primary poster here at YGM2026*), we have developed an experimental system in *S. cerevisiae* haploids to select for, quantify, and genomically characterize clones that carry HCC rearrangements. The process begins through the induction of a CRISPR-Cas9 DSB near the end of chromosome VI (Chr6), with a nearby repetitive sequence that is used in the HCC process. Depending on the nucleotide distance between the site of the DSB and the repetitive HCC substrate, 5'-3' resection may lead to the formation of a short or a long non-homologous ssDNA tail. We asked whether the removal of this ssDNA tail by Rad1-Rad10 endonuclease is required for HCCs to proceed and whether the length of the tail is also a factor. We used two different CRISPR/Cas9 guide RNAs to induce DSBs either 4bp or 92bp away from the repetitive region on Chr6, and measured the frequency of HCC formation in wild type cells and in *rad1Δ* mutants. When the DSB was made near the repeat (short 4nt tail), the frequency of HCC was the same between the two genotypes. However, with the longer tail (92nt), we observed a significant ~6-fold decrease in HCC frequency in *rad1Δ* mutants compared to wild type. We conclude from these results that the activity of Rad1-Rad10 is required when breaks occur far from a homologous sequence.

141B

Investigating the Role of SAW1 in DNA Repair Pathways in Saccharomyces cerevisiae

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Double-strand DNA breaks are a major threat to genome stability and are repaired through multiple pathways, including homologous recombination and single strand annealing (SSA). In *Saccharomyces cerevisiae*, Rad1–Rad10 is a structure-specific endonuclease that is required to specifically cleave recombination intermediates containing double-strand/single-strand (ds/ss) DNA junctions with 3' ssDNA. This Rad1-Rad10-dependent step is required for 3' non-homologous tail removal (3' NHTR) during SSA and gene conversion. *In vivo* and *in vitro* data have demonstrated that Saw1 is specifically required to recruit Rad1–Rad10 to 3' nonhomologous tails through interactions with Msh2–Msh3 and DNA, yet it remains unclear how these interactions are mediated and regulated *in vivo*. Rad1-Rad10 is also required for nucleotide excision repair (NER), which removes and repairs DNA damaged by UV light. Rad1-Rad10 is specifically recruited to NER intermediates by Rad14. To characterize Rad1-Rad10-Saw1 interactions and understand the role of Saw1 in recruitment of Rad1-Rad10 in 3' NHTR, we used a new cryo-EM structure of Rad1-Rad10-Saw1 as a guide to construct mutations in *SAW1* that we predict will disrupt Rad1-Rad10-Saw1 protein–protein interactions. We disrupted two regions of Saw1 that are predicted to make specific contacts with Rad1. We tested these mutations in different DNA repair pathways, including SSA assays, mating type switch (gene conversion) assays, and UV sensitivity assays to understand the *in vivo* role of Saw1 during specific DNA repair pathways. These mutations were compromised in double strand break repair pathways including SSA (3′ NHTR) and gene conversion. These experiments clarify how Saw1 coordinates Rad1–Rad10 activity and provide new insight into the DNA repair pathways and their mechanisms.

143B

Mutations in orc subunits and tfiia suppress the lethal phenotype of an orc atpase mutation

Luis Martinez[1], Stephen P. Bell[2] [1]Biology, Massachusetts Institute of Technology, [2]Biology, Massachusetts Institue of Technology

In eukaryotes, the origin recognition complex (ORC) selects origins of replication and promotes the loading of the Mcm2-7 replicative helicase complex at these sites, with the help of two additional proteins, Cdc6 and Cdt1. Five of the six ORC subunits are related to the AAA+ family of ATPases. Although functions for ATP hydrolysis by Cdc6 and the Mcm2-7 complex have been described, the essential role of ORC ATP hydrolysis remains unclear. We performed a genetic screen in *Saccharomyces cerevisiae* for suppressors of the lethal phenotype of the *orc4-R267A* allele, which disrupts ORC ATP hydrolysis *in vitro*. We identified six causative mutations, five of which were distributed across different ORC subunits. The suppressor mutations in Orc1

and Orc4 increased the *in vitro* helicase loading activity of the ATPase-defective ORC (ORC4R), whereas the remaining ORC subunit mutations did not improve the mutant protein's biochemical activity. Allele specificity studies revealed that most of the alleles specifically suppress defects in ATP binding and hydrolysis at the Orc1-Orc4 interface. The sixth allele is a mutation in TOA2, a subunit of the TFIIA complex. The *toa2* allele suppresses genetic defects at the Orc1-Orc4 interface. Mutations in the general transcription factors, TBP and TFIIB, and the large subunit of RNA Polymerase (RNA Pol II) also suppress the lethal phenotype of the *orc4-R267A*, suggesting that reducing transcription is sufficient for suppression. Our study identifies multiple ways to suppress the lethal phenotype of an ATPase defective ORC allele and reveals a connection between ORC ATP hydrolysis and transcription.

144B

Schizosaccharomyces octosporus Cdc24 complements *Schizosaccharomyces pombe cdc24* mutant

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The gene *cdc24+* plays a vital role in DNA replication in *Schizosaccharomyces pombe*. Although Cdc24 has does not have a sequence homolog in other eukaryotes, it does interact with highly conserved eukaryotic replication proteins. To investigate the evolutionary conservation of Cdc24 we have used comparative genomics and protein alignments to assess the degree of similarity of Cdc24 among related fission yeast. Upon determining that *S. octosporus cdc24+* is most comparable to *S. pombe cdc24+*, we constructed a plasmid to express *S. octosporus cdc24+* in an *S. pombe* background to observe *if S. octosporus cdc24+* can rescue function in *S. pombe cdc24* mutants. Using in-fusion ligation-independent cloning, we isolated six independent recombinants. Some of the recombinants were able to rescue ts lethality of both the *cdc24-M81* and *cdc24-G1* alleles, while others demonstrated allele specificity. Sequencing of the recombinants have revealed some new mutations which may highlight important functional portions of the Cdc24 protein. We report that *S. octosporus* Cdc24 is able to rescue loss of *cdc24* function in *S. pombe*, supporting that Cdc24 function is highly conserved among fission yeasts.

145B

The role of chromatin remodeling complexes in ploidy maintenance and genome integrity

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The integrity of genomes requires the faithful segregation of the newly replicated chromosomes during cell division. The main goal of this work is to understand 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 *S. cerevisiae* 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 (INO80C and SWR1C). Both ATP-dependent chromatin-remodeling complexes participate in a variety of biological processes including transcription, DNA repair and DNA replication. INO80C catalyzes the eviction of the H2A.Z histone variant replacing it with H2A in nucleosomes. This complex is comprised of 15 subunits, and their specific contribution to chromosome segregations remains largely unknown. The INO80C has been implicated in the maintenance of ploidy through the characterization of mutations in the genes encoding the Ies6 and Ino80 subunits (Chambers et al. doi:10.1101/gad.199976.112), which result in ploidy increase. The SWR1C catalyzes the exchange of H2A for H2A.Z. The yeast SWR1C 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 Ts alleles of *ARP4* and *SWC4,* for benomyl sensitivity, ploidy maintenance and chromosome segregation. Remarkable, these mutants show ploidy increase at semi-permissive temperatures. Furthermore, we analyzed genetic interactions among *ino80Δ* and *swr1Δ,* the catalytic subunits of both complexes. Although both individual mutants increase ploidy, *ino80Δ swr1Δ* double mutant maintains the ploidy increase behavior of *ino80Δ* mutants. This finding suggests that INO80C may have additional functions to ensure proper chromosome segregation, or that there are parallel ways to deposit H2A.Z. Importantly, the Ino80 and Swr1 subunits associate with pericentric chromatin and their presence correlates with the expected H2A.Z turnover. 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 the essential ones.

146B

To transpose or not to transpose, that is the question: the role of nitrogen in host susceptibility to Ty retrotransposition

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We previously observed that *Saccharomyces cerevisiae* evolved under nitrogen limitation experience retrotransposon (Ty1/2) derepression, which can give rise to novel insertions that often drive adaptation. Here, we demonstrate that most of those events take place in the last phase of growth (after terminal cell density is reached), and, in accordance with prior reports, supplementing adenine in medium reduces the observed number of transpositions to a similar level as observed under glucose limitation. Others have shown this phenomenon is likely due to diminished *de novo* synthesis of AMP. However, here we show that nitrogen starvation has consequences for host susceptibility to retrotransposition beyond what can be accounted for by limited adenine alone.

For the growth cycle immediately following stationary phase, previously nitrogen-starved cells exhibit a temporary "poised to transpose" state when returned to growth in medium that that does not repress nitrogen catabolism: even when adenine is supplemented. This "poised" state is observed where increased transposition is triggered when cells are plated with a poor and unfavored nitrogen source (proline) within a short window following return to growth. This temporary increased susceptibility to transposition is not observed if cells are starved for glucose and then switched to media with poor nitrogen content, or if nitrogen–starved cells are shifted into media with rich nitrogen content. Currently, we are evaluating the molecular basis of this "poised" state, whether this mechanism is host-adaptive, and if this transient state corresponds to a stage in the retroelement life cycle.

147B

Ligase-dependent and independent functions of the C-terminus of Mms21 contribute to optimal growth and genome stability in *Saccharomyces cerevisiae*

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Multiple molecular mechanisms that safeguard the fidelity of the genome are orchestrated by sumoylation, a process in which E1, E2 and E3 SUMO ligases facilitate the covalent linkage of SUMO peptide to a target protein. Mutations or mis-regulation in the SUMO pathway have been identified in genetic diseases characterized by genome instability. An evolutionarily conserved E3 SUMO ligase, Mms21, is one such example. Mms21, as part of the Smc5/6 complex, sumoylates proteins involved in DNA replication, tolerance or damage repair. Given the diverse roles of Mms21 in DNA metabolism, it is highly probable that Mms21 promotes genome stability through mechanisms independent of the ligase domain. To address this question, we examined the C-terminal truncation mutant of *Saccharomyces cerevisiae* Mms21, analogous to a mutant identified in a rare human condition characterized by genome instability. The human mutation C-terminally truncated the Mms21 protein, without affecting the residues in the E3 ligase domain. Based on multiple sequence alignments, we generated two mutants: *mms21Δ22* and *mms21Δ18,* lacking the last 22 amino acids and the last 18 amino acids, respectively. Truncating the last 22 amino acids of yeast Mms21, but not 18 amino acids, mimicked the human diseased mutation. *mms21Δ22* mutants exhibited more pronounced growth defect and DNA damage sensitivity than the wild-type and two well-characterized mutants of Mms21 – one with two missense mutations in the enzymatic domain and another without the entire enzymatic domain and the C-terminus. Furthermore, *mms21Δ22* mutants exhibited a G2/M delay during normal growth. In addition, Rad53 checkpoint was not fully activated in *mms21Δ22* mutants. Phenotypes of *mms21Δ22* mutants cannot be attributed to the reduced Mms21 protein levels as a result of the truncation – overexpressing the *mms21Δ22* allele did not rescue the phenotypes of *mms21Δ22* mutants. Our genetic data suggested that the C-terminus contributed to both ligase-dependent and -independent functions of Mms21 and opposed the activity of the adjacent domain, thereby fine-tuning genome integrity. The *mms21Δ22* disease allele analog further enhanced our understanding of Mms21's functions beyond its ligase activity in genome instability conditions.

148B

Kinetochore-microtubule attachments are strengthened by CENP-T stabilization of Stu2 at kinetochores

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Accurate chromosome segregation requires kinetochores to form robust, load-bearing attachments to dynamic spindle microtubules. Two competitive receptors recruit a major microtubule-binding complex, Ndc80c, to the kinetochore: Dsn1 and Cnn1 (CENP-T). Previously, we discovered that kinetochore components that co-purify with the yeast Dsn1 protein can maintain microtubule attachments under force. However, it was unclear whether yeast Cnn1 purifications can maintain load and whether Cnn1 makes distinct contributions to kinetochore function. Using an optical trapping-based force assay, we show that kinetochore particles purified via Cnn1 sustain dynamic microtubule attachments under load. Mutation of a conserved region within the disordered N-tail of Cnn1 significantly weakens attachment strength *in vitro* and results in a growth defect when Dsn1 function is compromised. The mutation also reduces Stu2 kinetochore levels without altering and restoring Stu2, either by direct addition *in vitro* or by tethering it to Ndc80c *in vivo*, rescues both attachment strength and cellular viability. Together, our findings reveal a biophysical role for Cnn1 in enabling Stu2-dependent stabilization of kinetochore-microtubule attachments and identify an additional feature of Stu2 interaction with the kinetochore

149B

Dissecting the molecular mechanisms controlling telomere-length homeostasis in *S. cerevisiae*

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Telomeres require the dedicated replication mechanism provided by the telomerase RNP complex. Without this activity, chromosomes erode, ultimately leading to genome instability, senescence, and death. A critical step in telomere-length maintenance is the preferential recruitment of the telomerase RNP to short telomeres. How short telomeres are sensed and how telomerase is recruited to them remain unclear. Additionally, the senescence program induced by critically short telomeres is poorly understood. The Counting Model proposed by the Shore and Blackburn labs involves Rap1-interacting factors (Rifs) being "counted" on telomeres to convey length to the negative-feedback telomerase-regulating system. But how are Rif proteins counted, and how do they inhibit telomerase action? We discovered that telomerase is recruited by Ku binding to Sir4, and that, since Rif1/2 compete with Sir4 for binding the same site on the telomeric DNA-binding Rap1 protein, Rifs' inhibition of telomerase could simply be by blocking Ku-Sir4 recruitment (Hass and Zappulla, *eLife* 2015; Chen *et al*., *Cell* 2018). Thus, we propose the RIVERSET model (Rif Inhibition versus Recruitment of Sir4 for Extension by Telomerase), which builds upon the Counting Model by including a reversible switch-like mechanism that toggles between extendibility states. Some of the strongest support for RIveRSET is that deleting the Ku-binding hairpin in telomerase RNA (TLC1) to abolish the Ku-Sir4 telomerase recruitment pathway suppresses the telomere hyperlengthening seen in *rif* mutants. Furthermore, we are now using a new approach to test the RIVERSET model built upon an inducibly linearizable minichromosome. This TeME (Telomerator Minichromosome Extendibility) assay allows us to control many aspects of the telomeric DNA revealed upon cutting. Using TeME, we are determining the optimal telomeric repeat length for telomerase extension and the upper threshold for the set point of telomere length homeostasis in wild-type and mutant strains. Beyond the scope of the RIVERSET model, we propose an overall three-step molecular-mechanistic model that integrates the Sir4-Ku and essential Est1-Cdc13 telomerase recruitment and activation functions. Thus, we are developing a molecular-mechanistic understanding of how telomere length is maintained by telomerase in *S. cerevisiae*.

150A

Identifying Wolbachia Effector Genes in Yeast

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Wolbachia are endosymbiotic bacteria that infect between 30 and 60% all insect species as well a number of filarial nematodes. They naturally infect *Drosophila* species and thus provide a model system for studying mechanisms of endosymbiosis. In addition, *Wolbachia* are of interest for their

connections to disease. They are obligate endosymbionts of several parasitic worms and reduce transmission of several RNA viruses (*e.g.*, zika and dengue) by mosquitos. Genetic studies of fruit flies have revealed host determinants of *Wolbachia* infections. However, because *Wolbachia* cannot be cultured independently of their hosts, we know little about *Wolbachia* effectors proteins necessary for endosymbiosis. We aim to identify *Wolbachia* genes required for endosymbiosis by expressing candidate Wolbachia effectors in budding yeast. Our strategy is to use Golden Gate cloning to place codon optimized Wolbachia genes under the control of the *GAL1* promoter and express them in yeast. Gene function is tested by monitoring growth on a variety of selective media. We will describe the design of the projects and our initial results. In addition, we will describe how this project can be adapted for teaching principles of molecular biology and genetics to high school students and undergraduates.

151A

Using Educational Outreach to Discover Multiple Genetic Pathways Towards Fungal Adhesion

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Evolution of fungal adhesion is visually dramatic, medically significant, and we can research it in high school classrooms. By selecting for *S. cerevisiae* adhesion using simple protocols, students witness evolution in real time while generating data relevant to pathogenesis, transmission, industrial biofilm formation, and ecological fitness. Classroom experiments serve as robust replicates of university-performed selections under varied conditions, enabling identification of pervasive evolutionary patterns across diverse environments. Our pilot experiments in a university lab reveal multiple genetic pathways to adhesion and, prior to this conference, students at two schools will have completed beta tests of our protocol.

Using strains engineered to produce colorful pigments, we performed pilot selections for adhesion to diverse surface materials. We developed a five-week evolution protocol that yields striking visible adhesion phenotypes. Whole genome sequencing revealed material-specific genetic changes. Parallel phenotypic assessments of flocculation and agar invasion similarly demonstrated substrate-dependent outcomes. Yeast evolved on bamboo frequently harbored SNPs in transcriptional regulators of flocculation, particularly CYC8 (11 of 13 populations), and exhibited pronounced agar invasion. In contrast, yeast selected for adhesion to hair, plastic, or glass often carried SNPs in ergosterol biosynthesis genes, suggesting altered membrane hydrophobicity as an alternative adhesion mechanism. Across all materials, most evolved populations displayed increased flocculation, and mutations clustered within discrete ORF regions, pointing to specific functional domains that warrant further characterization. Further pilots are underway to investigate adhesion to additional medically relevant substrates.

Through this five-week protocol, high school students observe evolution in real time while contributing to publishable research. Evolved strains undergo whole genome sequencing, and students analyze results using software developed specifically for high school use. We also created pedagogical materials that guide students in designing short follow-up experiments with clear visual outputs. Student pre- and post-assessments will measure gains in conceptual understanding and scientific identity. Together, these classroom experiments can reveal the breadth of mechanisms underlying fungal adhesion evolution while deepening student engagement with evolutionary biology.

152B

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 many tools and features making it indispensable for researchers. SGD engages in a variety of online training and educational outreach efforts to inform our users of new developments, to improve familiarity with features and tools, and to increase public awareness of the importance of yeast for both biological research and instructional purposes.

The Educational Resources page located within SGD's help documentation (https://sites.google.com/view/yeastgenome-help/general-science-resources/educational-resources) provides users with a venue for accessing and sharing information relevant to education in the biological sciences. This includes information about associations, specific classroom materials (teaching modules and project-based courses), references that provide information on projects suitable for undergraduates, and some sites of general interest to the aspiring biologist. To inform the community about features and tools, SGD has created and posted short videos to YouTube (https://www.youtube.com/SaccharomycesGenomeDatabase) to educate users and address their questions. This includes videos tools like: GO Term Finder, GO Slim Mapper, JBrowse, Variant Viewer, 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 microPublication (https://www.micropublication.org/) to promote the publication of brief, novel, technically sound research results 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 and Europe PMC for greater visibility.

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

153B

BUDDY: A Web Platform for Student-Led Inquiry and Functional Modeling of Human Clinical Variants in *Saccharomyces cerevisiae*

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The expansion of next-generation clinical sequencing is generating a massive accumulation of variants of uncertain significance, mutations whose impact on protein function and human health remains undetermined. While machine learning tools offer variant effect predictions, experimental data remains the gold standard. *Saccharomyces cerevisiae* (yeast) can serve as a powerful model for assessing functional effects that result from conserved variants; however, the requisite bioinformatic workflow can present a time and knowledge barrier. To address this, we developed BUDDY (Bioinformatic Utility for Diagnostic Discovery in Yeast), a user-friendly web app designed to streamline the functional modeling of human variants in yeast.

BUDDY utilizes a software pipeline that retrieves and maps human variants (ClinVar), identifies orthologs (DIOPT), aligns protein sequences, determines established yeast phenotypes (SGD), calculates local homology scores, and integrates pathogenicity predictions (AlphaMissense). Additionally, the platform automates the design of oligonucleotides needed for performing CRISPR/Cas9 genome editing. Visualization capabilities include an interactive 3D structural viewer that superimposes protein structures from both human and yeast.

The software also utilizes multimodal generative AI to assess the biological utility of modeling the chosen variant-gene pair, including using computer vision to assess the 3D protein overlay. Furthermore, AI is used to generate a safety-conscious experimental protocol for functionally evaluating the variant.

A primary objective of this platform is to facilitate student involvement in research, including through Course-based Undergraduate Research Experiences (CUREs). To reduce the number of lab sessions needed, current efforts include developing a single-step (cloning-free) streamlined CRISPR genetic engineering approach.

The platform provides versatile search capabilities, allowing students to conduct autonomous searches for keywords, browse genes associated with rare diseases, or execute more focused inquiries under the guidance of an instructor or mentor. We are currently testing BUDDY's real-world utility by examining how first-year medical students use it to develop experiments. By empowering novice researchers to model human variants in yeast, this platform democratizes functional genomics and integrates clinical relevance into educational settings.

154A

Glycolytic inhibitors converge on α-arrestin-dependent glucose transporter endocytosis

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Cancer cells undergo metabolic reprogramming known as the Warburg effect, favoring glycolysis even under aerobic conditions. This increases cellular reliance on glucose for energy production, making glycolytic inhibitors promising anti-cancer agents. However, resistance to these inhibitors frequently arises through mechanisms that remain poorly understood.

Our previous work in *Saccharomyces cerevisiae* defined the cytotoxic mechanisms and resistance pathways of 2-deoxyglucose (2DG), a toxic glucose analog. Building on these findings, this study investigates whether resistance mechanisms established for 2DG are conserved across other glycolytic inhibitors, including 3PO and 3-BrPA.

Dose-response assays established effective inhibitory concentrations in yeast for each glycolytic inhibitor. We then compared long-term growth of 2DG-sensitive and -resistant strains across inhibitors to assess conservation of resistance phenotypes. Acute responses were evaluated by monitoring localization of the glucose transporter Hxt3-GFP following treatment. While long-term assays revealed both similarities and differences among inhibitors, acute assays demonstrated a conserved response: 2DG, 3PO, and 3-BrPA all induce rapid Hxt3 endocytosis. Consistent with prior findings, 2DG-resistant mutants stabilized Hxt3 at the plasma membrane, while sensitive and wild-type strains rapidly internalized the transporter — a pattern mirrored under 3PO and 3-BrPA treatment. We previously showed this process is regulated by the parologous α-arrestins Rod1 and Rog3, and here we demonstrate that α-arrestin-dependent Hxt3 trafficking is similarly induced by 3PO and 3-BrPA, despite these drugs inhibiting glycolysis by distinct mechanisms. Complementation assays further identified Rod1 as a key regulator of Hxt3 internalization across multiple glycolytic inhibitors.

Together, these results reveal a conserved mechanism of action shared by structurally and mechanistically distinct glycolytic inhibitors. By defining shared and distinct resistance pathways, this work clarifies how cells adapt to metabolic stress and supports the development of more effective anti-cancer combination therapies.

155A

An INQuisitive study of Cellular Sequestration: The role of HSP42 in Nuclear Aggregate Formation

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The fitness of a cell is best reflected by the state of the proteome, as proteins are the workforce of the cell. When there are stressors that disrupt the proteins in the cell, they must enlist a multifaceted response to control the damage as otherwise they risk cell death. When proteins get disrupted, they can misfold and accumulate into protein aggregates, which are known to be involved in common diseases such as Alzheimer's and Parkinson's. Protein Quality Control (PQC) pathways are important in this regard, as they help remodel the proteome by refolding, disaggregating, and degrading misfolded proteins. One aspect of PQC that is underexplored is sequestering of the misfolded proteins to various cellular inclusions. Interestingly, yeast cells under various forms of stress will form a structure within the nucleus called the INtranuclear Quality control site (INQ) which also hosts various endogenous PQC

machinery that is involved in various forms of DNA damage repair. Sequestration to INQ is promoted by molecular chaperones Hsp42 and Btn2 under DNA damage. Our preliminary data has also shown that yeast strains with deletions of either Hsp42 or Btn2 cannot form INQ under stress conditions. The goal with this project is to characterize the pathways that Hsp42 and Btn2 act upon, as well as identifying and understanding the PQC machinery that is sequestered to INQ. By domain-hacking Hsp42, I aim to narrow down the roles that the domains play in the sequestration of disaggregated and endogenous proteins, as well as the localization to the nucleus. I hypothesize that nuclear protein sequestration is an adaptive mechanism to maintain cellular fitness during DNA damage across species. Further insight into these mechanisms could clarify the questions surrounding these debilitating diseases.

156A

Multi-omic investigations into isobutanol stress in *Saccharomyces cerevisiae*

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Ethanol has been a key component to the U.S.'s modern energy infrastructure, adding $53 billion to the U.S. GDP in 2024. Despite how crucial ethanol is as a biofuel to modern economies, its highly hygroscopic and volatile nature makes it a suboptimal additive to petroleum-based fuels. Due to these complications from ethanol, isobutanol (IBA), a next-generation biofuel, is a superior replacement that mitigates these concerns. As with ethanol, sugars can be fermented into IBA by using microbes, such as *Saccharomyces cerevisiae* (*S. cerevisiae*), as a chassis for generating the fusel alcohol. Unfortunately, IBA is known to have relatively high toxicity in *S. cerevisiae*, which is expected to impose a barrier to producing high titers. To better understand and begin to overcome this toxicity, we performed multi-omic experiments and analyses on the proteome, transcriptome, and metabolome on IBA-challenged *S. cerevisiae*. GO enrichment analysis suggests that *S. cerevisiae* may be responding to DNA damage and disruptions in replication, transcription, and protein-folding during IBA exposure. These results may provide candidate genes to improve IBA tolerance and biofuel production, as well as helping identify fundamental alcohol stress mechanisms in eukaryotic biology.

157A

The E2 ubiquitin conjugase Rad6 regulates energy homeostasis in yeast

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The E2 ubiquitin-conjugating enzyme Rad6 is a pleiotropic regulator best known for its role in transcriptional control through the monoubiquitination of histone H2B (H2Bub). Loss of H2Bub has been linked to carbohydrate metabolism and the establishment of a metabolic program compatible with respiration. In addition, emerging evidence suggests that Rad6 may also influence mitochondrial physiology. However, the role of Rad6 in energy homeostasis remains unclear. Here, we uncover a novel role for Rad6 in regulating the transition between metabolic states using *Saccharomyces cerevisiae* as a model system. By integrating transcriptomic and proteomic analyses, we found that loss of Rad6 shifts cells into an intermediate metabolic state with features of both fermentation and respiration. Notably, these features are present in glucose-rich conditions in which yeast cells would typically favor fermentation. Our biochemical and functional analyses of *rad6Δ* cells revealed upregulation of post-exponential phase and stress response genes, improved growth in non-fermentable carbon sources, and increased reliance on mitochondrial activity for growth, without major changes in mitochondrial abundance. Targeted metabolomic profiling of *rad6Δ* cells showed increased levels of tricarboxylic acid (TCA) cycle intermediates and associated amino acids compared to the wild-type strain suggesting increased activation of the TCA cycle. Consistently with that, glucose tracing experiments demonstrated higher incorporation of labeled carbons into TCA cycle metabolites, indicating enhanced mitochondrial carbon flow from glucose. These metabolic adaptations suggest that *rad6Δ* cells are primed to respire even in fermentable carbon sources, indicating a malfunctional capacity do adjust to the glucose levels. To investigate the molecular basis of this phenotype, we performed ubiquitin remnant (KGG) proteomics to profile global ubiquitination changes. Loss of Rad6 resulted in widespread remodeling of the ubiquitin landscape, with enriched changes in proteins involved in metabolic pathways, including fatty acid metabolism and mitochondrial function. Taken together, our findings support a model where Rad6-dependent ubiquitination drives change in activity of metabolic substrates as well as their degradation, leading to a global metabolic rearrangement that bias cells to a respiration-prone metabolic state. This work expands the functional scope of Rad6 beyond chromatin regulation and highlights ubiquitination as a central mechanism in metabolic adaptation.

158A

Redox regulation of spatial dynamics and assembly of the sequestrase Hsp42 in yeast

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Molecular chaperones undergo post-translational modifications at specific amino acids under various conditions that determine their relevant functions. These modifications have been interpreted to constitute a type of chaperone code. Under oxidative stress, the chaperone code involves the oxidation of cysteine or methionine residues, leading to changes in chaperone function, such as converting a foldase into a holdase. While the redox chaperone code of the Hsp70, Hsp90, and nucleotide exchange factor families have been extensively studied, the small heat shock proteins have not received the same attention. Therefore, we focus on the oxidation of the small heat shock protein in the model organism budding yeast. Previously, we found that redox-challenged cells lacking thioredoxin reductase (*trr1*Δ) activate the heat shock response and induce the hyperaccumulation of the small heat shock protein/sequestrase Hsp42 with misfolded proteins. Building on that finding, this study reveals that cysteine 127 (C127) of Hsp42 is redox-active and becomes oxidized in *trr1*Δ cells, forming a disulfide bond that contributes to Hsp42 oligomerization. This disulfide bond formation at C127 was confirmed by a cys-to-ser mutation and SDS-PAGE with and without reducing agents. Additionally, we detected Hsp42 C127 oxidation in wild-type (WT) cells using divinyl sulfone (DVSF) crosslinking. The apparent molecular weight of the oxidized form of Hsp42 observed under non-reducing SDS-PAGE is three times

the size of the monomer form. Mass spectrometry analysis of this high molecular-weight band indicates that it consists solely of Hsp42, suggesting it is a novel trimer-like homo-oligomer. Interestingly, when the prion-like domain (PrLD) at the N-terminus is deleted, the trimer-like form is not detected; instead, a dimer form is observed. This result suggests that the PrLD domain contributes to the formation of the oxidized Hsp42 oligomer. Taken together, our data demonstrate that the small heat shock protein Hsp42 can sense redox signaling through the oxidation of cysteine 127, which subsequently influences Hsp42 oligomerization and may affect its sequestrase ability. Further analyses are needed to examine in detail how C127 oxidation triggers oligomer formation, as well as how it affects Hsp42-protein client interactions and foci stability.

159A

Allocation of transcriptional and translational capacity

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Ribosome biogenesis is an energic intensive requirement of cells and is tightly linked to the growth rate. Ribosomal proteins comprise the bulk of the cellular proteome and ribosomal rRNA is 80% of total RNA in the cell. Therefore, as cells anticipate reduction in nutrients, ribosome biogenesis is the first pathway that is down regulated in cells. While RNA Pol II transcribes mRNAs including all ribosomal protein genes (RPGs), RNA Pol I is solely responsible for the transcription of three of the four ribosomal RNAs (rRNAs). The production of the RPGs and rRNAs must be coordinated. Perturbation in either process reduces long-term survival as translational capacity suffers. In an effort to determine the genetic basis of the regulation of translational capacity, a genetic screen of the *S. cerevisiae* knockout out was carried out with a construct that increased translation nearly 1000-fold. Many candidate enhancers and suppressors were identified. As cells starve, TORC1 activity decreases leading to increase in autophagy and decrease in ribosome biogenesis. Reducing translational capacity makes cells extremely sensitive to TORC1 inhibition by rapamycin. Future experiments will assess the role of these candidates in regulating translational capacity. These processes are highly conserved and foundational to all cellular growth. These pathways often become dysregulated during cancer, and the results of these studies will contribute to understanding of how cells regulate energy intensive processes.

160A

Atg41 and the chromatin remodeling complex RSC are essential for ribophaghy in S. cerevisiae

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During nitrogen (-N) starvation, macroautophagy (autophagy) is activated to recycle precursors and help cells cope with nutrient stress. We used the auxin-inducible degron (AID) strategy to identify chromatin remodeling complexes that regulate autophagy. We report that RSC is a positive regulator of autophagy. RSC is an essential chromatin remodeling complex in *S. cerevisiae* that stimulates transcription by evicting or repositioning histones. Depleting the RSC ATPase subunit (Sth1) led to a 60% reduction in GFP-Atg8 processing in -N, an indication of reduced autophagy flux. N starvation induces both bulk and selective autophagy. In yeast, only two factors are known to promote selective degradation of ribosomes via autophagy (ribophagy): 1) the deubiquitination complex Bre5/Ubp3, which leads to only 60S ribosome degradation and 2) Rpl12A, a recently identified ribophagy receptor, required for degrading both 40S and 60S subunits. To test if RSC promotes ribophagy, we analyzed GFP-tagged ribosomal proteins degradation upon Sth1 depletion in -N. *STH1-AID* cells exhibit robust ribophagy induction, evidenced by free GFP accumulation by western blot. However, this accumulation was largely abolished upon Sth1 depletion; for example, the levels decreased by 96% in Rps6A-GFP (40S) cells and by 87% in Rpl12A-GFP (60S) cells. These findings firmly implicate RSC as a ribophagy-promoting complex. To determine whether RSC acts through Atg11, a selective autophagy adapter, we deleted *ATG11* in the *STH1*-AID background. No significant change in GFP accumulation was observed in *atg11*Δ, making Atg11 dispensable for ribophagy. To understand how RSC promotes ribophagy, we performed Pol II and H3 chromatin immunoprecipitation (ChIP) to identify *ATG* genes whose expression is regulated by RSC. We found that *ATG41*induction in -N was associated with an 80% drop in promoter histone occupancy, which was eliminated upon depleting Sth1. The Pol II occupancies also declined significantly. Moreover, *ATG41* is regulated by Gcn4; however, Sth1-depletion did not reduce Gcn4 binding. Thus, RSC promotes transcription of *ATG41* by remodeling its promoter chromatin after the binding of Gcn4 to the *ATG41* promoter. Finally, we asked if Atg41 promotes ribophagy. Strikingly, in the *atg41*Δ cells, free GFP accumulation of both large and small ribosomal subunits declined by 80-90%, indicating that Atg41 is a critical regulator of ribophagy. In comparison, *atg41*Δ reduced GFP-Atg8 processing by 30%, suggesting a major role in ribophagy. Given that Atg41 colocalizes with Atg9 in -N, it is plausible that Atg41 delivers ribosomes for degradation to phagophores. Together, our study identifies a chromatin-dependent regulatory step in ribophagy. We show that RSC-mediated induction of *ATG41* is crucial for the selective degradation of ribosomes in nitrogen starvation, and also implicate Atg41 as a primary driver of ribophagy in yeast.

161A

Histone H3K4 Methylation Gates PP2A Signaling to Regulate ER Redox Adaptation During Oxidative Folding Stress

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Cells adapt to environmental and metabolic stress by redistributing signaling activities across intracellular compartments, yet whether chromatin state governs this spatial reorganization remains unknown. Here we show that histone H3 lysine 4 methylation (H3K4me) gates stress-induced phosphatase redistribution. Loss of H3K4 methylation activates a cytoplasmic PP2A–Sch9 signaling axis that selectively enhances endoplasmic reticulum (ER) oxidative folding when disulfide-bond formation becomes limiting. This response operates independently of canonical unfolded protein response-mediated transcription and TORC1 inhibition and instead relies on post-translational redistribution of PP2A signaling between nuclear and cytoplasmic compartments. Phosphoproteomic and functional analyses identify dephosphorylation of Sch9 as a key effector linking chromatin state to metabolic rewiring and ER redox adaptation, thereby promoting cellular fitness. These findings establish histone methylation as a regulator of signaling compartmentalization, enabling rapid organellar adaptation through post-translational control rather than transcriptional remodeling.

162A

Nuclear elongation in the *Saccharomyces cerevisiae* mating response requires expression and proper localization of the inner nuclear membrane protein Prm3

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The budding yeast *S. cerevisiae* response to their mating pheromone entails cell elongation (shmooing) prior to cell membrane fusion, as well as nuclear elongation prior to nuclear fusion (karyogamy). The mechanisms driving nuclear elongation, which is under the control of the transcriptional regulator of the mating response, Ste12, have remained poorly understood. We found that pheromone-induced nuclear elongation is a result of accelerated expansion of the nuclear membrane relative to nuclear volume. We further observed that nuclear elongation is dependent on de novo fatty acid synthesis, which is not required for cell elongation. To find factors responsible for nuclear elongation, we screened for Ste12-induced genes whose deletion resulted in reduced nuclear elongation in response to a-factor mating pheromone. Two genes were identified, encoding respectively the transcriptional regulator Kar4 and the nuclear membrane protein Prm3. While Prm3 was known to be required for membrane fusion during karyogamy, its role in nuclear elongation was novel. Moreover, Prm3's role in nuclear elongation is distinct from its role in karyogamy, as deletion of Kar5, which mediates Prm3's requirement in karyogamy, is not required for nuclear elongation. To map the functional domains of Prm3, a series of partial deletions were examined, revealing a 24-residue region whose deletion yielded reduced elongation as well as mislocalization from the nuclear membrane to the ER. A fusion of a foreign bipartite NLS to this mutant restored localization and nuclear elongation and nuclear elongation, suggesting that (a) Prm3 is itself an inner nuclear membrane (INM)-anchored protein, (b) that the 24-residue region serves as a bipartite NLS, and (c) that INM localization is essential for PRM's role in nuclear elongation. Moreover, deletion of a subset of this region disrupted nuclear elongation without disrupting nuclear fusion, further suggesting these processes are separable. Because of the link between lipid synthesis and nuclear elongation, we hypothesize that Prm3 may, when localized to the INM, promote expansion of the nuclear membrane, potentially by inducing lipid synthesis. Changes in lipid profiles in response to mating pheromone are consistent with this possibility. The role of nuclear elongation in the mating process and the mechanism by which Prm3 induces nuclear elongation are under investigation.

163A

The effects of glyphosate based-herbicides on the yeast metabolome and mitochondrial function

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First marketed as Roundup®, glyphosate-based herbicides are among the most widely used herbicides worldwide. Glyphosate is an inhibitor of the shikimate pathway, which produces chorismate. Chorismate is essential for the production of aromatic amino acids and, even though the pathway is absent from animals, there is increasing evidence of glyphosate toxicity in various animal models and humans. *Saccharomyces cerevisiae* is a useful eukaryotic model to elucidate the molecular mechanisms underlying glyphosate toxicity. The standard laboratory strain S288c is sensitive to glyphosate exposure and previous work from our lab has shown that *DIP5*, a glutamate/aspartate permease, is a key player in glyphosate uptake. *DIP5* deletion mutants exhibit resistance to the pesticide, likely because Dip5 facilitates glyphosate transport into the cell owing to its structural similarity to its canonical substrate, glutamate. Our previous work has also shown that glyphosate affects the expression of *AAC1* and *HFA1*, genes associated with mitochondrial function. In this study, we developed a rapid LC-MS metabolomics pipeline to investigate the effects of glyphosate exposure on the yeast metabolome. We found that cells lacking *DIP5* have significantly lower levels of intracellular glyphosate compared to wild-type, confirming that Dip5 is essential for the import of glyphosate. Our metabolomic analysis showed a disruption in nitrogen metabolism as reflected by amino acid levels and urea cycle-associated metabolites in wild-type cells. Supplementation with aromatic amino acids allowed yeast to bypass shikimate pathway toxicity and revealed a disruption in glycolysis, gluconeogenesis, and central carbon metabolism pathways. We then directly measured oxygen consumption in cells exposed to glyphosate and found a significant defect in respiration in wild-type cells, but not in cells lacking *DIP5* or the mitochondrial glutamate transporter genes *AGC1* and *YMC1*. Additionally, we observed a complete inability of yeast cells to grow on acetate as a sole carbon source in the presence of glyphosate, further supporting the hypothesis that their mitochondrial function is severely impaired. Our findings suggest that glyphosate toxicity extends beyond the shikimate pathway and provide important insights into off-target effects, particularly mitochondrial function.

164B

Phosphorylated Gβ: a key role player in yeast gradient sensing

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Mating yeast is a powerful model of G-protein-coupled receptor (GPCR) mediated chemotropism: Haploid yeast cells detect a pheromone gradient secreted by their mating partner via a GPCR on their surface, triggering the mating response. However, how cells decode the shallow, complex, and dynamic pheromone gradient to orient toward their partners remains poorly understood. We have proposed that yeast decode pheromone gradients by assembling a gradient-tracking machine (GTM), comprising signaling, trafficking, and polarity proteins, at a default polarity site. Then the GTM redistributes up the pheromone gradient and stabilizes at the chemotropic site. Gβ links the receptor to the rest of the GTM components through its direct interaction with scaffold protein Far1. Pheromone induces Gβ phosphorylation on multiple sites. Multiple positive feedback loops that amplify the intracellular gradient signal depend on the local accumulation of phosphorylated Gβ ($G\beta^{P}$). Using a $G\beta^{P}$ biosensor, we showed that $G\beta^{P}$ is a leading marker that localizes to the upgradient side of the GTM. We also observed that active Cdc42, which ultimately positions vesicle delivery, accumulates as a patch on the upgradient side of the GTM. Since Gβ recruits the Cdc42 GEF (Cdc24) via its interaction with the Far1-Cdc24 complex, we postulated that $G\beta^{P}$ positions Far1 upgradient within the GTM. To test this possibility, we used a phospho-minus form of Gβ, $G\beta^{3TA/SA}$. In $G\beta^{3TA/SA}$ cells, Far1 was depolarized and positioned at the middle within the GTM, compared to WT cells where Far1 was polarized and positioned far upgradient within the GTM. Reduced Sec3 polarity in $G\beta^{3TA/SA}$ cells coincided with reduced tracking. These findings suggest another positive feedback loop that contributes to intracellular signal amplification. Furthermore, the "lysis during fusion" phenotype observed in $G\beta^{3TA/SA}$ cells is consistent with a defect in cell wall remodeling. We have shown that Gβ recruits active Rho1, an essential component of cell wall remodeling, to the polarized growth site. To ask whether Gβ-$Rho1^{GTP}$ plays a role in gradient sensing or fusion, we imaged a reporter of $Rho1^{GTP}$ in mating yeast. We found that Gβ and $Rho1^{GTP}$ colocalized during GTM assembly, tracking, and stabilization. It remains to be determined whether $Rho1^{GTP}$ plays a role in gradient tracking or is merely carried to the fusion site by the GTM. Altogether, this study establishes $G\beta^{P}$ as a key regulator of yeast gradient sensing.

165B

The yeast mitochondrial Porin represses Snf1/AMP Kinase signaling to attenuate viral replication

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Although fungi are broadly infected with mycoviruses, the antiviral mechanisms fungal cells use to oppose viral replication are not well understood. Here we discover a new mitochondrially controlled signaling mechanism in the budding yeast *Saccharomyces cerevisiae* that limits replication of L-A, an RNA mycovirus that endemically infects this organism. We show that Por1, the mitochondrial voltage dependent anion channel, prevents hyper-replication of L-A in stationary phase cells that have exhausted media nutrients. By investigating known stationary phase regulators, we find that deletion of the AMP-activated Kinase homolog *SNF1* reverses hyper-replication of L-A observed in *por1*Δ cells. This epistatic relationship suggests that Por1 negatively regulates Snf1 in stationary phase cells and derepressed Snf1 promotes L-A hyper-replication. We confirm this model, first demonstrating that *POR1* prevents the accumulation of activated Snf1 throughout stationary phase. By investigating Snf1 signaling targets we show that this *POR1-SNF1* regulatory mechanism acts in stationary phase cells to limit amino acid availability that sustain L-A replication. *POR1-SNF1* signaling represents a novel physiological control mechanism to limit viral replication in a eukaryotic cell.

166B

Examining the role of the hexosamine biosynthetic pathway in stress tolerance of the *S. cerevisiae pgm2*Δ mutant

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In *S. cerevisiae*, the enzyme phosphoglucomutase (PGM) facilitates the conversion between glucose-1-phosphate (Glc-1-P) and glucose-6-phosphate (Glc-6-P). Studies have shown that strains lacking the major isoform of this enzyme (*pgm2Δ*) develop a variety of defects when grown on galactose-containing media. Further studies demonstrated that loss of *SPT4* suppresses several stress-associated phenotypes in the *pgm2Δ* mutant; however, deletion of *SLT2* resulted in lethality that could not be rescued by removal of *SPT4*. Although low concentrations of glucosamine, which enhances the hexosamine biosynthetic pathway (HBP), have been shown to partially rescue *slt2Δ* sensitivity, glucosamine supplementation failed to suppress the lethality of the *pgm2Δslt2Δ* mutant on galactose. Recent studies have identified the HBP, regulated through Slt2-dependent signaling and *GFA1* expression, as an important metabolic support pathway during endoplasmic reticulum (ER) stress. Based on these findings, this study aimed to examine whether increasing HBP input through glucosamine (GlcN) supplementation could support growth of *pgm2Δ* strains when unfolded protein response (UPR) signaling is disrupted. Furthermore, this study focused on determining whether the previously observed *spt4Δ*-mediated rescue persists under these conditions. To address this, *pgm2Δhac1Δ*, *pgm2Δire1Δ*, and corresponding *spt4Δ* combinations, were plated on galactose-containing media supplemented with 5.25 mM or 7.5 mM GlcN, with and without an ER stress inducer. All strains exhibited reduced growth, and no rescue was observed at either glucosamine concentration. Under ER stress conditions, growth defects were further exacerbated. Notably, *spt4Δ* failed to rescue in any condition and instead displayed lethality in the presence of glucosamine. Additionally, this study aimed to investigate whether overexpression of *GFA1* in *pgm2Δ*, *pgm2Δspt4Δ*, *pgm2Δslt2Δ*, *pgm2Δhac1Δ*, and *pgm2Δire1Δ* backgrounds could enhance HBP activity and support survival under stress to define the limitations of glucosamine-mediated metabolic rescue and further clarify the role of HBP signaling in maintaining viability of stress-compromised *pgm2Δ* strains.

167B

Nuclear Expression of podoATP9-5 in Yeast Elicits a mitoCPR Response

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In the beginning of eukaryote evolution, the proto-mitochondria was formed from the endosymbiosis of a sulphur purple alphabacteria into an archaeal host. Modern mitochondria require ~1000 different proteins to function. Nonetheless, the ancient mitochondrial genome, encoding thousands of genes, was pared down such that current mitochondria genomes encode at most ~70 proteins across all eukaryotic lineages, and in yeast, specifically, only 6 proteins. Reduction of ancient mitochondrial genome resulted from gene loss of nonessential genes, or otherwise the transfer of essential mitochondrial-encoded genes to currently be encoded within the nuclear genome.

Our interests lie in characterizing the evolutionary mitochondria-to-nuclear gene transfer. Gene transfer likely initiated with a gene duplication event yielding a copy in the mitochodrial genome and the second copy in the nucleus. Completion of gene transfer would have required maintaining the nuclear gene copy at the expense of the loss of the mitochondrial copy. For retention solely of the nuclear copy, a number of hurdles must have been navigated that the protein encoded by the mitochondrial gene copy did not have to encounter. For example, the nuclear-encoded protein must be targeted to the mitochondria, localized to the appropriate submitochondrial compartment, and also not be mistargeted to other organelles.

To model this gene transfer process, we are studying the *ATP9-5* gene from *Podospora anserina*. *podoATP9-5* expressed from the nucleus is able to rescue the mitochondrial defect in the absence of mitochondrial-encoded *ATP9* gene of S. cerevisiae (Bietenhader et al, 2012). We have determined that high-gene dosage of *podoATP9-5* was toxic to yeast growth. Transcriptome analysis revealed that *podoATP9-5* expression elicited a mitoCPR transcriptional pathway response. This suggested that the nuclear-expressed podoAtp9-5 protein, although able to rescue the *sc-atp9-null* phenotype, was clogging protein trafficking in the mitochondrial inner membrane.

168B

Differential Tolerance for SEA Domain Misfolding Encodes a Mechanism for Mucin-Dependent MAPK Specificity

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Transmembrane mucins function as environmental sensors for Mitogen Activated Protein Kinase (MAPK) pathways. In yeast, the same mucin regulates different MAPK pathways controlling filamentous growth (fMAPK) and the response to osmotic stress (HOG) through specificity mechanisms that remain unclear. Using AlphaFold predictions, we found Sea urchin sperm protein, Enterokinase, Agrin (SEA) domains in yeast and other fungal mucins that are conserved in human mucins and other signaling glycoproteins. Functional analysis identified SEA domain variants that selectively impair individual MAPK pathways. Variants defective for fMAPK pathway signaling showed defects in protein folding, resulting in altered mucin levels, retention in the secretory pathway, and enhanced turnover by endoplasmic reticulum-associated degradation (ERAD). Remarkably, most variants retained function in the HOG pathway, revealing differential tolerance to SEA domain misfolding as a mechanism for HOG pathway activation and MAPK pathway specificity. Specificity was also conferred by the mucin cytosolic tail, which was required in the fMAPK pathway but was dispensable for the HOG pathway. Changes in the folding properties of protein domains may be a general way to transmit information, particularly about environmental stress.

169B

Examining the Effect Metal Toxicity has on Vacuole Inheritance and Structure in Saccharomyces cerevisiae

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Our research investigates how toxic metals, including lithium, magnesium, copper, and iron, affect vacuole function and structure in Saccharomyces cerevisiae. The yeast vacuole, a membrane-bound organelle, is crucial for maintaining cellular homeostasis, metal detoxification, degradation, and storing metals, phosphates, and selected amino acids. In S. cerevisiae, the vacuole shares homology with the mammalian lysosome with regards to degrading cellular waste. Our lab discovered lithium inhibits the inheritance of the vacuole from the mother cell to the daughter bud. Under lithium-free conditions, GSK3 phosphorylates Yck3, which subsequently phosphorylates Vps41. Phosphorylation of Vps41 reduces its affinity for vacuole fusion, thereby promoting proper vacuole inheritance. Lithium is proposed to inhibit GSK3, preventing this phosphorylation cascade and disrupting vacuole inheritance. We tested this putative pathway by taking knockout strains of genes in this pathway, and testing whether lithium continued to exert an effect on vacuole inheritance. Deletion of Yck3 caused ~50% inheritance defects in buds under lithium and control conditions, while Vps41 deletion reduced this defect to ~15%. Furthermore, introduction of a VPS41-phosphomimic plasmid into the vps41Δ strain lowered the inheritance defects to ~2%. However, it was unknown whether the phosphorylation of Vps41 alone restores inheritance in the absence of Yck3. To address this, we transformed the yck3Δ strain with the VPS41-phosphomimic plasmid, subjected the cells to lithium treatment, and used microscopy to quantify the percentage of defective inheritance in mother-daughter pairs to exhibit the loss of inheritance. These experiments revealed that phosphorylation of Vps41 alone did not restore inheritance without Yck3, suggesting that vacuole inheritance may be regulated in a more complex, non-linear manner. This also suggests that Vps41 may not be the most downstream effector in the proposed GSK3-Yck3-Vps41 pathway and additional intermediate proteins or pathways may play a more central role in facilitating inheritance. By clarifying the molecular mechanisms of vacuole inheritance, this work expands our understanding of organelle dynamics in eukaryotic cells under stress conditions.

170B

The Bar1 α-factor protease is critical for cell-autonomous gradient sensing in mating yeast

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The mating response of the budding yeast *S. cerevisiae* relies on chemotropism, a fundamental cellular process. Haploid cells of opposite mating types detect pheromone gradients produced by one another and polarize their growth towards the gradient source. To locate potential mating partners, cells assemble a gradient tracking machine (GTM) composed of sensory, polarity, and secretory proteins at default polarity sites. The GTM then incrementally redistributes upgradient along the cell membrane until it reaches the region of maximal external pheromone concentration. This process is called gradient tracking. While signaling mechanisms are known to amplify the intracellular signaling gradient to enable gradient tracking, it is unclear whether cells can steepen the external pheromone gradient. *MAT***a** cells secrete Bar1, an α-factor protease critical for mating efficiency. We have shown that *bar1*Δ cells are not only unable to orient toward potential mating partners but are also defective in mating at the default polarity sites, suggesting that gradient sensing is required even when moving the polarity site is not. Bar1 is thought to have both population-wide (global) and local (cell-autonomous) effects on partner detection and mating fidelity. We hypothesize that secreted Bar1 steepens the external α-factor gradient along the GTM to enable pre-morphogenic gradient tracking. To test this, we asked whether Bar1 released globally by wild type cells could rescue gradient tracking by *bar1*Δ cells. We found that global Bar1 rescued the ability of *bar1*Δ cells to default mate – perhaps by preventing receptor saturation at the default site – but not to gradient track. The inability of *bar1*Δ cells to track despite exogenous Bar1 indicates that locally secreted Bar1 enables gradient tracking through a cell-autonomous mechanism. We reasoned that the cell-autonomous effect of Bar1 would require its directed secretion by gradient-tracking cells. Indeed, we found Bar1 secretion was highly polarized at the cell periphery in response to α-factor treatment. Together, our results are consistent with Bar1 providing a cell-autonomous gradient-steepening effect to enable accurate pre-morphogenic gradient sensing.

171B

A cellular atlas of spatial protein quality control

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Cells constantly experience proteotoxic stress. To survive, they must properly fold proteins and maintain their integrity in the crowded cellular environment. Failures in proteostasis can lead to cellular dysfunction, cell death, and organismal disease. As a result, organisms have evolved chaperones and clearance mechanisms to protect the integrity of their proteomes. A key component of protein quality control (PQC) is the sequestration of misfolded proteins into

membraneless compartments, which limits further aggregation and facilitates reactivation or clearance by the ubiquitin proteasome system (UPS) and autophagy-lysosome pathways. In budding yeast during stress, misfolded proteins in the cytosol and nucleus rapidly form small inclusions. These inclusions actively traffic through the cell and, in prolonged stress, coalesce into three main inclusions: the intranuclear quality control compartment (INQ) in the nucleus and the juxtanuclear quality control compartment (JUNQ) and insoluble protein deposit (IPOD) in the cytosol. However, much about spatial PQC, including the chaperone dependence of different inclusions, the factors required for clearance, and the endogenous clients, as well as how these processes are coordinated on a cellular level, remains poorly understood.

To more deeply understand these processes, we developed an APEX2-based proximity labeling method to map the interactome of spatial PQC substrates in yeast. For the first time, we systematically identify key components of spatial PQC pathways in both the nucleus and the cytosol, including chaperones, ubiquitin ligases and other UPS elements, and membrane trafficking pathways. Additionally, we identify potential endogenous substrates of these compartments, including regulatory factors that may aid in coordinating the response to proteotoxic stress at a cellular level, broadening our understanding of the physiological roles of these PQC pathways. This research offers new insights into the cellular management of misfolded proteins and lays the groundwork for future studies on spatial PQC regulation and the molecular basis of proteostasis failures in aging and neurodegeneration.

172B

Studying the Mechanism for Ribosome-Inactivating Protein-Induced Growth Suppression Daniel Judge, Jennifer SO Gallagher West Virginia University

Ribosomes play a fundamental role in cell growth and cell cycle progression by synthesizing the proteins necessary to maintain homeostasis and respond to external stimuli and growth cues. This study focuses on a specific class of proteins called ribosome-inactivating proteins (RIPs), a category of potent toxins, such as ricin and sarcin, that irreversibly damage the large ribosomal subunit rRNA through an adenine N-glycosidic cleavage referred to as depurination. While the role of the catalytic subunit of ricin, known as ricin toxin A chain (RTA), is well-established, the precise process linking ribosome damage to altered cell morphology remains unclear. The ultimate result of ricin poisoning in mammalian cells is apoptosis. However, ricin toxicity does not significantly affect yeast cell viability. Instead of directly killing yeast cells, ricin slows down cell cycle progression, thereby increasing individual cell lifespan, but at the same time drastically inhibiting bud formation, which is necessary for reproduction and colony propagation. In this study, we engineered a tetracycline-regulatable expression vector to express RTA in *S. cerevisiae*. Using growth assays in both solid and liquid media, we characterized the effects of RTA expression on yeast growth, viability, and reproductive potential. Additionally, we quantified RTA transcript and ribosome depurination levels after induction. We further examined the effect of knocking out candidate genes involved in ribosome recycling and the unfolded protein response, including *hac1Δ*, *ire1Δ,* and *hbs1Δ,* to determine how these pathways affect growth after ribosomal depurination. To expand on this, high-throughput genetic screens were conducted using the full MAT alpha knockout collection to identify elements that increase or decrease sensitivity to RTA poisoning. Long-term, this research aims to provide a comprehensive understanding of the cellular pathways affected after exposure to RTA and establish a controlled method for reliably inducing apoptosis in specific target cells. This information can further research in cancer biology, embryology, and immunology, providing new mechanisms that can be explored to better understand homeostasis and how cells respond to stress.

173A

Molecular mechanisms that link mitotic exit to cell growth Sarah Beth Avila[1], Beth Prichard[1], Giovanni Guerra[2], Erin Jeffs[1], Andrea Gallardo[1], Doug Kellogg[1] [1]Molecular, Cell and Developmental Biology, University of California, Santa Cruz, [2]Biochemistry, University of Washington

In all cells, cell growth is required for cell cycle progression. The mechanisms by which cell growth controls the cell cycle remain poorly understood. In previous work, we found that growth controls mitotic progression in budding yeast via a signaling network known as the mitotic exit network (MEN). Two key components of the MEN are Net1, an rRNA transcription factor, and the phosphatase Cdc14. In a canonical model, Net1 binds and inhibits Cdc14 in the nucleolus; phosphorylation of Net1 releases Cdc14 into the cytoplasm, where it drives mitotic exit. Here, we discovered growth-dependent signals that control both Net1 and Cdc14. In addition, our results suggest that Net1 plays a much more direct role in promoting mitotic progression than previously thought, while Cdc14 is more likely an inhibitor of Net1. Together, these discoveries establish molecular links between cell growth and cell cycle progression and suggest an entirely new perspective on the function and regulation of the MEN.

174A

QTL Mapping Reveals the Genetic Architecture of Yeast Life History Traits Dominick S Costanzo, Dimitra Aggeli, Gregory Lang Biological Sciences, Lehigh University

Most phenotypes are complex, with many different loci throughout the genome contributing to the overall trait in a quantitative manner. A powerful strategy for uncovering the loci contributing to complex traits is Quantitative Trait Loci (QTL) mapping, where in two yeast strains are crossed and the resulting segregants are sequenced and phenotyped. We used a large mapping population (~100,000 segregants) from a cross between a laboratory strain and a vineyard isolate to map traits associated with yeast life history, including cell-cycle progression and mating efficiency. Individual segregants were barcoded and genotyped to allow for bulk assaying of the segregants. We identified more than 150 loci associated with yeast mating efficiency and cell cycle progression, as well as evidence of tradeoffs, particularly regarding progression through the cell cycle. Using composite interval mapping, we identify loci with sizes ranging from a few thousand kb to over 100,000 kb. Between experiments we find similar loci, which could indicate tradeoffs between traits. We find that the percent variance explained for cell cycle progression is quite low (~3%) compared to mating efficiency (~13%), likely due to the high complexity of these traits. We identified and validated candidate genes underlying QTL by selecting two loci of interest, *IRA2* and *HBT1* to reconstruct and verify their effects in our assays. We find that *HBT1* and *IRA2* both have the expected effect direction in mating efficiency, though the effect size, especially of *HBT1*, is diminished.

175A

Domestication drives repeated evolution of sexual-asexual life cycle trade-offs in yeast

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For thousands of years, humans have domesticated animals and cultivated crops by managing reproduction and selecting for desirable traits. In contrast, microbial domestication has often occurred unintentionally, and the variation of life cycle as well as its impact on genome evolution remain poorly understood. Here, we systematically examined life cycle variation across a diverse panel of 771 diploid *Saccharomyces cerevisiae* isolates from both wild and domesticated lineages. We identified widespread alterations in sexual reproduction, including impairments of sporulation, spore viability, and mating-type switching. These changes led to the emergence of two distinct life cycle strategies, favoring either asexual or sexual reproduction, which were notably enriched in domesticated clades. Haplotype analyses of the *HO* mating-type switching gene revealed multiple, independent loss-of-function mutations, indicating convergent evolution of heterothallism. While a preference for sexual life cycle often correlated with increased genomic heterozygosity in domesticated and human-associated clades, this relationship was not uniform across all lineages. We propose that the co-occurrence of altered sexual and asexual cycle preferences results in a trade-off that balances outcrossing and the subsequent maintenance of heterozygosity in domesticated populations. Finally, we provide a CRISPR-based molecular toolbox and a stable haploid strain collection spanning global genetic diversity, enabling further genetic research and industrial applications.

176B

A yeast adhesin-based surface display platform for the rapid assessment of amyloid aggregation

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Adhesins are fungal cell wall-associated proteins required for wild-type filamentous growth and flocculence. Notably, nearly 90% of identified adhesins contain sequences possessing high amyloid-forming potential. The *Saccharomyces cerevisiae* flocculin Flo11 is a canonical adhesin with predicted amyloidogenic regions between amino acids 1036 and 1245. Data suggest that this sequence contributes to cell flocculence, and, here, we investigated the role and modularity of the adhesin amyloid-forming region in affecting flocculence. Our data indicate that a protein fragment corresponding to the predicted Flo11 amyloidogenic sequence indeed forms an amyloid structure *in vitro*, with characteristic dimensions by electron microscopy and positive staining with thioflavin T. To determine if the Flo11 amyloid region is sufficient to modulate flocculence, we constructed a synthetic adhesin scaffold consisting only of the Flo11 GPI anchor sequence with and without its amyloid-forming region. We introduced this plasmid into a non-filamentous strain of *S. cerevisiae* deleted of *FLO11* and found that presence of the amyloid-forming region within this construct increased cell-cell adhesion by flocculence assays. Conversely, mutation of the sequence significantly decreased flocculence. Further, the adhesin amyloid region is modular. We swapped the Flo11 amyloid-forming sequence in this adhesin surface display scaffold for amyloid-forming regions from other fungal adhesins. Sequences from the *S. cerevisiae* adhesins Aga1, Fig2, and Flo1 and the *Candida albicans* adhesins Hwp1, Hwp2, Rbt1, and Sap1 increased flocculence relative to the *flo11* deletion strain carrying an adhesin construct lacking any amyloid-forming region. Moreover, amyloid-forming sequences from human Tau, Aβ peptide, and a-synuclein support flocculence in place of the *FLO11* sequence in the adhesin construct above. We generated selected point mutants of the 40-amino acid Aβ peptide from amyloid precursor protein and found that a single substitution of alanine for Ile32 and Met35 significantly decreased yeast flocculence relative to wild type Aβ. Consequently, we view this Flo11-based surface display system as an effective platform for the rapid determination of amyloid aggregation, applicable to eukaryotic and metazoan sequences, with yeast flocculence as a simple readout sensitive to single amino acid substitution.

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Molecular mechanisms that link cell cycle entry to cell growth

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The decision to enter the cell cycle in late G1 phase is a critical event that occurs only when cells have undergone sufficient growth in early G1 phase. The key molecular event that initiates cell cycle entry is expression of late G1 phase cyclins. However, the mechanisms that initiate expression of late G1 phase cyclins, and how they are linked to cell growth, remain unknown. To better understand how cell growth drives cell cycle entry, we searched for growth-dependent signals that influence expression of late G1 phase cyclins. We discovered that membrane trafficking events that drive plasma membrane growth are required for expression of late G1 phase cyclins. We further discovered that a conserved MAP kinase cascade likely plays a critical role in the mechanisms that drive growth-dependent entry into the cell cycle. Together, these discoveries suggest the existence of novel and likely conserved mechanisms that link cell cycle entry to cell growth.

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BacTrack: a high-efficiency, global method for mutagenesis and phenotypic analysis

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The current cutting-edge for genetics is genome-wide screens that allow assessment of phenotypes of thousands of mutations in parallel. Budding yeast is an excellent system for such large-scale screens because of its haploid genome and because it shares many cellular processes with higher eukaryotes. Whereas traditional screens employ mutagens to induce random mutations throughout the genome, such mutations are difficult to track in pooled cultures. While the yeast knockout collection provides a complete survey of the genome and easily traceable mutations, these strains are prone to compensatory mutations, require specialized infrastructure, and are only available in one genetic background. To overcome these limitations, we have developed a high-efficiency transposon mutagenesis method called BacTrack that offers the flexibility of traditional screens combined with readily traceable mutations. The

highly active plasmid-borne piggyBac transposon bearing a selectable marker allows selection for transposon insertion into the genome. Mutants are created and assessed on-demand in a pooled manner, allowing rigorous assessment of relative fitness effects using next generation sequencing.

Using BacTrack, we have identified synthetic lethal interactions with Nup2—a nuclear porin that is necessary for physical interactions between active genes and the nuclear pore complex, a co-activator of transcription. Additionally, we have screened for interactions with histone methylation mutants H3K4A and Set1Δ to identify interactions that differ between the two and potentially point to a role for Set1 beyond its methyltransferase activity.

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Biochemical Pathways at SGD: Comprehensive Integration and Access Across Multiple Platforms

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The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) provides extensive biochemical pathway information through multiple integrated platforms, enabling researchers to access pathway data in diverse formats tailored to specific research needs. Central to this ecosystem is YeastPathways (pathway.yeastgenome.org), SGD's comprehensive metabolic pathway database built on the Pathway Tools framework, which encompasses over 200 curated pathways including metabolic, biosynthetic, and degradation routes. YeastPathways features interactive pathway diagrams, enzyme details, and metabolic network visualization tools that support hypothesis generation and experimental design. Pathway annotations extend beyond YeastPathways through Noctua (noctua.geneontology.org), the Gene Ontology Consortium's pathway modeling platform where SGD curators contribute detailed GO-CAMs (Gene Ontology Causal Activity Models) that represent molecular mechanisms as structured networks of causal relationships. These models capture protein activities, their regulatory connections, and biological contexts with unprecedented granularity. On SGD gene pages, pathway information appears in multiple contexts: direct links to YeastPathways for genes encoding metabolic enzymes, Gene Ontology annotations describing biological processes, and connections to Alliance of Genome Resources (www.alliancegenome.org) GO-CAMs that integrate cross-species pathway knowledge. This multi-layered presentation ensures researchers encounter pathway data regardless of their entry point. For computational users, SGD offers downloadable pathway datasets in BioPAX format, enabling programmatic access and integration with pathway analysis tools, network modeling software, and systems biology applications. Pathway-relevant GO annotations are also available through standard GO annotation file downloads (sgd-archive.yeastgenome.org/curation/literature/), supporting enrichment analysis and functional genomics workflows. *SGD is funded by the US National Institutes of Health, National Human Genome Research Institute (NHGRI) [U24HG001315]. Our efforts are also supported via the Gene Ontology Consortium (GOC) [U41HG002273] and the Alliance of Genome Resources [U24HG010859].*

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High-resolution, meiosis-free mapping of genetic variation with CRI-SPA-Map

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Genome sequence variation contributes to phenotypic trait differences, but the exact genes and variants influencing trait values remain largely unknown. Linkage limits the resolution of traditional genetic mapping approaches. We have developed CRI-SPA-Map, a strategy that combines CRISPR, selective ploidy ablation (SPA), and high-throughput phenotyping to increase the resolution of genetic mapping without relying on meiotic recombination.

CRI-SPA-Map utilizes a donor Saccharomyces cerevisiae W303 strain with a SPA cassette near each centromere and a plasmid encoding CRISPR machinery targeting the commonly-used KanMX antibiotic resistance cassette. When this W303 donor strain is mated to a BY strain of the S. cerevisiae Yeast Knockout (YKO) collection in which a given gene is replaced with KanMX, the resulting diploid repairs a double-stranded break created in KanMX using the homologous chromosome from the donor strain. SPA is then induced to remove the donor genome and generate haploid BY strains that carry small stretches of W303 DNA at the location previously occupied by the KanMX cassette.

Whole-genome sequencing of 552 isolates derived from YKO strains harboring gene deletions on the left arm of chromosome XIV showed successful replacement of KanMX with W303 DNA. Repair tracts typically spanned between 4,878 and 14,082 base pairs with variable lengths observed even across isolates derived from the same YKO strain. Using phenotyping data from over 1,000 isolates, we mapped increased growth rate in nutrient-rich media to a region containing the MKT1 and SAL1 genes. This region was four times smaller than yeast growth quantitative trait loci identified with traditional genetic mapping approaches. We further dissected this region to two causal variants in MKT1 and SAL1 and revealed that these variants show epistatic interactions that depend on the growth environment.

To broaden the applicability of CRI-SPA-Map to strain backgrounds beyond the YKO collection, we used the PiggyBac transposon to create a library of strains with a single KanMX insertion. We randomly selected 9 insertion strains to use in the CRI-SPA-Map procedure. Whole-genome sequencing of the resulting CRI-SPA-Map isolates confirmed the PiggyBac insertion was seamlessly replaced with tracts of donor DNA. Therefore, the combination of transposon mutagenesis and CRI-SPA-Map creates new opportunities to fine map genetic variation at high resolution without meiotic recombination.

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Integration of *Saccharomyces* Genome Database Data into The Alliance of Genome Resources

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The *Saccharomyces* Genome Database (SGD; yeastgenome.org) has served as the premier resource for curated genomic and biological information about *Saccharomyces cerevisiae* for nearly three decades. As a founding member of the Alliance of Genome Resources (the Alliance; alliancegenome.org), SGD is actively integrating its comprehensive curated data and specialized tools into this unified platform to enhance cross-species comparative genomics and improve data accessibility across model organism databases (MODs). Core datasets from SGD have been successfully integrated in to the Alliance, including gene annotations, phenotype data, disease associations, and genetic and physical interaction data. The Alliance

now displays SGD-curated information alongside data from other model organisms, providing a harmonized, orthology-based view that facilitates translational research and cross-species comparisons. Tools in SGD that are common across multiple MODs, such as BLAST, Textpresso, and InterMine, have also been incorporated into the Alliance. We will continue to harmonize common datatypes and to migrate yeast-specific data into the Alliance. This integration will strengthen SGD's contributions to the broader research community and enhance the Alliance's capacity to support comparative analyses across diverse organisms. SGD is funded by the US National Institutes of Health, National Human Genome Research Institute (NHGRI), [U24HG001315]. Our efforts are also supported via the Gene Ontology Consortium (GOC) [U41HG002273] and the Alliance of Genome Resources [U24HG010859].

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Adaptive laboratory evolution of *Saccharomyces cerevisiae* reveals novel mechanisms of tolerance to plant toxins

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Plants have evolved many defense mechanisms to combat pathogenic fungi. One such mechanism is the production of anti-fungal secondary metabolites called saponins. Saponins have previously been found at increased levels in switchgrass (*Panicum virgatum*) grown in drought and drought-like conditions. *Saccharomyces cerevisiae* can be used to convert lignocellulosic feedstocks, such as switchgrass, into biofuels, but *S. cerevisiae* growth is inhibited in feedstocks exposed to drought. Currently, the mechanisms of inhibition due to saponins on *S. cerevisiae* are not well understood. Here, we performed Adaptive Laboratory Evolution experiment in switchgrass feedstocks with increasing levels of added saponin (protodioscin) and then sequenced promising evolved candidates for genes of interest conferring increased tolerance. From three separate flasks with the same starting parent strain, three distinct mutations in a transcriptional regulator of Pleiotropic Drug Response (PDR) efflux pumps, were identified, as well as distinct mutations within PKA pathway genes. We propose that these mutations in the PDR pathway alter the expression of drug efflux pumps to extrude saponins or other inhibitory compounds out of the cell. Understanding these mechanisms could lead to increased and more stable cellulosic biofuel production, potential antifungal targets, and the ability to harness *S. cerevisiae* to create saponins for other purposes.

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Fine-scale genetic dissection of yeast persistence in mice

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Identifying the genetic mechanisms that allow fungi to persist in mammalian hosts could ultimately enable better antifungal therapies. The budding yeast Saccharomyces cerevisiae is an opportunistic pathogen and provides a powerful model for studying fungal persistence in vivo. In earlier work, we used a cross between the laboratory reference strain BY4716 (BY) and the clinical isolate 322134S (3S) to map the genetic basis of yeast persistence across mouse organs, identifying 17 loci that explain most of the heritable variation in this trait. About half of these loci have consistent effects across organs, while the others show antagonistically pleiotropic effects, improving persistence in either the brain or non-brain organs but not both. A major challenge in moving from loci to mechanisms is that the segregants that persist best in mice often carry complex combinations of alleles across multiple loci. These loci cannot easily be dissected through additional crosses, because recombination would break apart the allele combinations that give rise to high persistence. To overcome this challenge, we are using a synthetic genomics strategy based on CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a method our lab developed for cloning and assembling segments of natural chromosomes. This approach will allow us to resolve the loci to their causal genes by synthetically recombining haplotypes of the loci in otherwise constant genetic backgrounds. Identifying these genes will reveal the molecular mechanisms that allow yeast to persist in mammalian hosts, both generally and within specific organs, and will provide a new framework for dissecting trait loci in yeast and other species.

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DNA prospecting at petascale identifies efficient PET degrading enzymes with novel properties

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Plastic waste has become a global crisis, with over 400 million tonnes produced annually since its widespread adoption in the mid-20th century. Enzymatic recycling—particularly via PET-degrading enzymes (PETases)—offers a promising solution, yet the full diversity and functional potential of these enzymes remain largely unexplored. Here, we present a scalable, discovery-driven approach to mine PET-degrading enzymes across unprecedented sequence space. Leveraging Logan (logan-search.org), the largest DNA database to date, we identified over 100 million putative A/B hydrolases, the enzyme family to which PETases belong. From this vast dataset, we selected 162 enzymes spanning the PETase phylogenetic landscape and reconstructed 20 ancestral homologs. Using a high-throughput yeast-based screening pipeline, we assessed activity *in vivo* and found that ~7.5% of extant homologs exhibited PET-degrading activity, compared to 30% of ancestral variants. Guided by these results, we implemented a targeted re-sampling strategy around high-activity clades, identifying six additional active enzymes and increasing the positive hit rate to 46%. Notably, functional characterization revealed unexpected enzymatic diversity: some enzymes displayed PET polymerization activity—a previously unreported function—while others matched the efficiency of the benchmark FAST-PETase in degrading a BHET substrate. Together, our results demonstrate a powerful framework for large-scale enzyme discovery and uncover previously hidden functional diversity within PETases, opening new avenues for efficient plastic depolymerization and biotechnological innovation.

185A

Genetic Minimization of *Saccharomyces cerevisiae* Chromosome I

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A central goal in biology is to understand the minimum set of genes required to produce a viable eukaryotic cell. Genome minimization, systematically reducing a genome to only the genes required for viability, offers a powerful way to approach this problem, but achieving this in eukaryotes remains a major challenge. Here we introduce MoSAIC (Minimal or Streamlined Architectures of Individual Chromosomes), a method for experimentally removing many non-adjacent regions from a target chromosome in the budding yeast Saccharomyces cerevisiae. MoSAIC leverages recombination between a native chromosome and synthetic DNA fragments to generate panels of euploid cells carrying substantially reduced versions of that chromosome. Applying this approach to Chromosome I, we constructed a strain harboring a dramatically streamlined version of the chromosome, retaining only 41% of the ORFs from that chromosome. Genome-wide transposon mutagenesis in this background shows that this minimized chromosome increases sensitivity to genetic perturbations both within and outside Chromosome I that are otherwise tolerated in wild-type cells, suggesting that many genes previously considered nonessential become functionally essential in this reduced genome. These results demonstrate the feasibility of large-scale chromosome minimization in a eukaryote while revealing biological constraints that will shape efforts to build a minimal eukaryotic genome.

186A

Mapping conditional genetic interactions of whole genome duplication paralogs in angiosperm-like niches

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The whole genome duplication (WGD) occurred in the ancestor of the yeast *Saccharomyces cerevisiae* 100 MYA. The emergence of angiosperms coincided with this WGD and resulted in a profound change in the ecosystem. 551 WGD paralog pairs have been retained and many are enriched in the carbohydrate metabolism, suggesting their importance in enabling the yeast fermentative lifestyle and conferring a selective advantage necessary to survive the increasingly sugar-rich environment provided by flowering fruiting plants. The selective advantage may come from the increased gene dosage, buffering due to functional redundancy or the specialization from functional divergence, but still remains poorly understood. We previously constructed a collection of 240 double gene deletion mutants and 480 corresponding single mutants of WGD paralogs and screened them for digenic and trigenic interactions using trigenic Synthetic Genetic Array (τ-SGA). While many paralogs could be classified as divergent or redundant, a third of pairs (79/240) showed sparse genetic interaction profiles, preventing their functional characterization. Since genetic interaction networks are modulated by environmental conditions, and this analysis has been performed in standard nutrient-rich media, it overlooked the natural ecological stresses in which yeast evolved. Here, we investigate the functional divergence of 79 paralog pairs using conditional complex genetic interaction analysis in environments that reflect the angiosperm niche. We used τ-SGA and measured colony size as a proxy for fitness of 158 single and 79 double mutants on 13 conditions comprising high sugar osmolarity (16%-28%), thiamine deficiency, mixed carbon sources (glucose, fructose and sucrose), acetic acid exposure (10 mM – 40 mM) and pH stress (3.0 and 3.8). We will select paralog pairs that show negative digenic interactions and will conduct conditional trigenic interaction analysis. We hypothesize that highly correlated digenic interaction profiles will reveal dosage paralogs, a higher ratio of digenic compared to trigenic interactions will reveal divergent paralogs, which specialized, whereas more trigenic compared to digenic interactions will reveal redundant paralogs with buffering relationships. This project aims to uncover conditional divergence and redundancy of WGD paralogs and shed light on the evolutionary mechanisms driving duplicated genes retention.

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Creating a large designer cellulosome in yeast to boost ethanol production

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Cellulosic biomass represents a promising feedstock for biofuel and biochemical production. However, its recalcitrant structure strongly hinders enzymatic degradation. Cellulosomes are large multi-enzyme complexes that are highly efficient at degrading cellulose, but all the cohesins in a native cellulosome are identical, so that the cellulase types and their positions in a cellulosome cannot be controlled. Here, we constructed the largest designer cellulosome known to date into *Kluyveromyces marxianus* to boost cellulose degradation efficiency. Using innovative techniques, we synthesized a designer CipA (cellulosome integrating protein A) gene that encodes nine distinct species-specific type I cohesins and two cellulose-binding modules, which we named SCipA2B9C. Then, we fused nine distinct fungal cellulases separately with nine distinct type I dockerins for their precise positioning on SCipA2B9C to achieve enzyme proximity-effect. We constructed three yeast hosts to compare their performances. First, an enzyme host (EH) secretes nine dockerin-fused cellulases, including endoglucanases (EgIII-a, EgIII-m and EgIII-c), exoglucanases (CBHII-j and EXG2-r), β-glucosidases (BGS-f and BGS-l) and cellulase boosters (LPMO-t and CDH-b). Second, the scaffoldin host (SH) expresses SCipA2B9C. Third, the cellulosome-9 host expresses SCipA2B9C and nine dockerin-fused cellulases. Native-PAGE and ELISA confirmed specific interactions between dockerins and cohesins. Additionally, native-PAGE, SDS-PAGE and LC-MS verified the successful assembly of the multi-enzyme complex. Our performance evaluation showed that co-culturing of EH and SH outperformed the cellulosome-9 host. It degraded microcrystalline cellulose efficiently to produce 14.29 g/L bioethanol, which surpassed all previously constructed yeast cellulosomes by fourfold or more. In summary, our study provides an effective approach to biomass degradation.

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Discovery of GPCR agonist antibodies with unique activation mechanisms using the yeast FAST Platform

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Antibodies are highly specific, avoiding off-target activities that challenge GPCR drug discovery. However, their pharmacological actions on GPCRs have been predominantly inhibitory. Abalone Bio's Functional Antibody Selection Technology (FAST) platform in yeast combines synthetic biology and machine learning (ML) to discover and design functionally active antibody drugs—such as agonists, positive or negative modulators, or antagonists. FAST goes beyond mere binding by simultaneously measuring the function of 100 million antibodies against GPCRs directly, allowing us to find rare antibodies that are hard to discover through traditional screening methods with limited throughput. Furthermore, the platform does not depend on structural information, which is limited for dynamic receptors like GPCRs, bypassing the challenge of predicting allosteric interactions from static structures. FAST couples GPCR function to cell growth in a quantitative and tunable manner, enabling sensitive identification of functional antibodies with a wide range of activity. These discovery campaigns produce library-scale sequence-function datasets that uniquely power generative protein language models to design novel active antibody sequences.Using FAST, Abalone Bio has identified multiple agonists against four GPCRs, including a Class B GPCR target.

Agonist antibodies specifically targeting metabolic class B GPCRs could reduce the GI side effects that limit current obesity drugs. Leveraging the FAST platform, we successfully identified agonist antibodies for this Class B GPCR and further optimized the antibodies by functional maturation. This functional maturation process focuses on enhanced functional potency and efficacy over binding affinity, diverging from conventional affinity maturation. This approach enabled the identification of novel agonists with robust preclinical performance.

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A Programmable T7 Bacteriophage Produced in Yeast for Custom DNA Delivery

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Viruses are extraordinarily efficient molecular machines for delivering DNA into cells, but their ability to replicate makes them difficult to harness safely, and their high natural specificity can limit their use in designer applications such as gene delivery and phage therapy. One way around these challenges is to assemble viral particles in an orthogonal host such as Saccharomyces cerevisiae, where viral components can be produced but the resulting particles can never replicate or spread. Prior work has shown that viral capsid proteins expressed in yeast can spontaneously self-assemble non-replicative Virus-Like Particles (VLPs), and even relatively complex systems such as Adeno-Associated Virus (AAV) have been reconstructed in yeast to deliver engineered DNA cargo. Building on this concept, we are developing ScT7 (Saccharomyces cerevisiae T7), a programmable bacteriophage platform assembled in yeast. The system is encoded on a 42 kb yeast neochromosome containing 21 codon-optimized genes from the E. coli T7 phage under the control of yeast promoters. These genes direct the self-assembly of phage particles capable of packaging up to ~40 kb of custom DNA cargo while lacking the genes required for replication. By modifying the gp19 tail fiber responsible for host recognition, these particles should be amenable to retargeting to different bacterial hosts, enabling large-scale DNA delivery for applications such as phage therapy, mitochondrial engineering, and microbiome engineering.

190B

Isoform-Specific Cytochrome b5 Enhances CYP87D20-Catalyzed Oxidation in Engineered Yeast for Cucurbitane-Type Triterpenoid Production

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Cucurbitacin B is a bioactive cucurbitane-type triterpenoid produced in *Cucumis melo* with potent anticancer and anti-inflammatory activities. However, its low natural abundance and structural complexity limit efficient production through plant extraction or chemical synthesis. In our previous study, the cytochrome P450 enzyme CYP87D20 was identified as a key catalyst involved in oxidative modification during cucurbitacin B biosynthesis, yet its catalytic efficiency remained low when expressed in *Saccharomyces cerevisiae*, suggesting that additional redox partners may be required for optimal activity. In this study, we reconstructed the upstream cucurbitacin biosynthetic pathway in *S. cerevisiae* using a CRISPR/Cas9-based engineering strategy and performed bioinformatic analyses to identify accessory proteins potentially associated with CYP87D20. A specific cytochrome b5 (CYB5) isoform was found to significantly enhance CYP87D20-mediated oxidation, leading to increased production of the intermediate 11-carbonyl-20β-hydroxycucurbitadienol. Notably, this enhancement was isoform-specific, as four additional CYB5 isoforms failed to produce comparable effects. These results reveal an isoform-dependent role of cytochrome b5 in modulating plant P450 activity and highlight the importance of compatible redox partners for efficient heterologous expression of plant metabolic pathways. The engineered yeast strains developed in this study provide a promising microbial platform for cucurbitane-type triterpenoid biosynthesis and facilitate further elucidation of the cucurbitacin B pathway.

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Genomic and Phenotypic Landscape of the Industrial Yeast *Cyberlindnera jadinii:* Ploidy Variation, Genetic Diversity, and Metabolic Potential

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Cyberlindnera jadinii (Torula yeast) is recognized for its robust growth under diverse stress conditions, yet its genetic architecture remains largely unexplored. To establish *C. jadinii* as a chassis for industrial biotechnology, we integrated whole-genome sequencing with high throughput phenotypic profiling across 20 wild strains. Our analysis revealed a population structure defined by ploidy and single nucleotide polymorphisms (SNPs). We identified a mixed population of diploid and triploid strains, where ploidy was the primary determinant of cell morphology. Triploids exhibiting significantly larger cell volume. Conversely, fitness traits across 24 distinct environmental conditions were driven by SNP-defined lineages rather than ploidy levels. Notably, *C. jadinii* harbors a nucleotide diversity ($\pi = 18 \times 10^{-3}$) four-fold higher than that of *Saccharomyces cerevisiae*, highlighting a substantial degree of natural variation within the species. Furthermore, we functionally characterized the *C. jadinii* homolog of *FPS1* (*CjFPS1*); its heterologous expression in *S. cerevisiae* significantly enhanced growth on glycerol and acetate, underscoring its conserved metabolic function in carbon utilization. These findings provide the first comprehensive view of the genotypic and phenotypic landscape of *C. jadinii*, underscoring its potential as a versatile platform for biotechnological applications.