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1. The structure of an Ndr/LATS kinase - Mob complex reveals a novel kinase-coactivator system and substrate docking mechanism. *Kyle Schneider[1], Gergõ Gógl[2], Brian Yeh[1], Nashida Alam[1], Alex Nguyen Ba[3], Alan Moses[3], Csaba Hetenyi[2], Attila Reményi[2],* ***Eric Weiss***[1]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Institute of Enzymology, Hungarian Academy of Sciences, Budapest; 3) Department of Cell & Systems Biology, University of Toronto.
Hippo signaling pathways are ancient in eukaryotes; they perform crucial functions in the control of cell proliferation and morphogenesis. In budding yeast, a form of hippo signaling called the RAM network controls the final event of mother/daughter separation and modulates polarized growth. In all hippo pathways, NDR/LATS kinases in complex with Mob coactivator proteins are key regulators of downstream target proteins. To understand how these enzymes are regulated and recognize in vivo substrates we solved the structure of the budding yeast Cbk1-Mob2 complex in three different crystal forms, providing the first structural template of an NDR/LATS kinase - Mob coactivator assembly. These structures suggest that Mob coactivator association allows NDR/LATS enzymes to use a novel adaptation of an AGC kinase activation mechanism that involves a highly conserved hydrophobic C-terminal motif. We have also discovered that the kinase domain of Cbk1 engages in a docking interaction with a short peptide motif present in its two known substrates, Ssd1 and Ace2, and have defined the surface of the kinase involved in this interaction. We find that the conserved docking motifs in Ace2 and Ssd1 enhance robustness of their control by Cbk1. Furthermore, examination of docking motif co-evolution with phosphorylation consensus motifs indicates that docking motif conservation strongly indicates that a protein is an in vivo Cbk1 regulatory target. This highlights an expanded set of seven high-confidence RAM network regulatory targets that form a functionally related group of effectors present at the site of cytokinesis. Analysis of a number of substrate orthologs across a wide range of fungi supports a sequential model for adaptive evolution of kinase docking, in which substrates first gain phosphorylation consensus sites and subsequently acquire docking motifs in otherwise rapidly changing unstructured regions.

2. Morphogenesis checkpoint kinase Swe1 is the executor of lipolysis-dependent cell cycle progression. ***Sepp D. Kohlwein****, Neha Chauhan, Myriam Visram.* Institute of Molecular Biosciences, University of Graz, Graz, Austria.
Cell growth and division requires the precise duplication of cellular DNA content, but also of membranes and organelles. Knowledge about the cell cycle-dependent regulation of membrane and storage lipid homeostasis is only fragmentary. Previous work from our laboratory has shown that the breakdown of triglycerides (TG) is regulated in a cell cycle dependent manner, by activation of the Tgl4 lipase by the major cyclin-dependent kinase Cdc28. The lipases Tgl3 and Tgl4 are required for efficient cell cycle progression at the G1/S transition and their absence leads to a cell cycle delay (Kurat et al., Mol Cell 2009). We now show that defective lipolysis activates the Swe1 morphogenesis checkpoint kinase and halts cell cycle progression by phosphorylation of Cdc28 at tyrosine residue Y19. Saturated long-chain fatty acids and phytosphingosine supplementation rescue the cell cycle delay in lipase deficient strains, suggesting that Swe1 activity responds to an imbalanced sphingolipid metabolism, in the absence of TG degradation.

3. Compartmentalization of G1/S regulators allows signaling information to traverse a switch-like transition. ***Andreas Doncic****, Jan M Skotheim.* Department of Biology, Stanford university, Stanford, CA.
The ability to specify and maintain discrete cell fates is essential for development. However, the dynamics underlying selection and stability of distinct cell types remain poorly understood. Previously, we provided a quantitative single-cell analysis of commitment dynamics during the mating-mitosis switch in budding yeast and showed that commitment to division corresponds precisely to activating the G1 cyclin positive feedback loop in competition with the cyclin inhibitor Far1. We showed that the coherent feed-forward regulation of the CDK inhibitor Far1 by the MAPK Fus3 integrates the pheromone signal to increase the stability of the pheromone-arrested state in response to a more persistent input signal. Here, we show how the decision to enter the cell cycle is enhanced by nuclear/cytoplasmic compartmentalization. Nuclear Far1 and Cln1/2 sets the point of commitment and the pool of nuclear Far1 is degraded before the cytoplasmic Far1 upon cell cycle entry. We show that the cytoplasmic pool of Far1 is stabilized in cycling cells exposed to low amounts of pheromone and thus provide daughter cells born in low pheromone conditions with a memory of the mother cell¹s pheromone exposure. Indeed, we find that cells inheriting more Far1 from their mother arrest longer. Thus, compartmentalization of similar pathway components allows distinct cytoplasmic and nuclear CDK thresholds that govern memory and decision-making functions respectively.

4. Coordination of cell cycle-regulated gene expression by Cdk1. *Benjamin Landry, Claudine Mapa, Heather Arsenault, Kristin Poti,* ***Jennifer Benanti***. Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA.
Accurate cell division depends upon the coordination of many cellular processes with the cell cycle. This coordination is

achieved in part by the orchestrated expression of groups of genes that peak in different cell cycle phases. Cyclin-dependent kinase (Cdk1) plays a key role in regulating this cyclical gene expression, and it phosphorylates almost all cell cycle-regulatory transcription factors (TFs). However, the details of how phosphorylation modulates the functions of many cell cycle-regulatory TFs are not well understood. Here, we find that simultaneous elimination of Cdk1-mediated phosphorylation of four S-phase TFs delays mitotic progression and reduces fitness of budding yeast. Although cell cycle-regulated genes still cycle in the absence of phosphorylation, peak expression of many S and M/G1 phase genes decreases, indicating that Cdk1 modulates gene expression levels. Blocking phosphorylation interfered with SCF-mediated degradation and stabilized each of the four TFs. Consistent with these findings, blocking phosphorylation of the repressors Yox1 and Yhp1 led to increased chromatin association and decreased expression of target genes. Interestingly, we found that Cdk1 coordinated the activation and degradation of the activator Hcm1. Phosphorylation of the C-terminus of Hcm1 promoted its association with target gene promoters, whereas phosphorylation of the N-terminus promoted its degradation. Altogether, we conclude that Cdk1 promotes late cell cycle gene expression by both activating transcriptional activators and inactivating transcriptional repressors. Furthermore, our data suggest that coordinated regulation of the TF network by Cdk1 is necessary for faithful cell division.

5. Understanding the Regulation, Composition and Function of P bodies and Stress Granules in Quiescent Cells. ***Khyati H Shah***, *Paul K Herman.* Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210.
In response to stress, eukaryotic cells accumulate mRNAs and proteins at discrete sites in the cytoplasm. Two of the best characterized of these granules, P bodies (PBs) and Stress Granules (SGs), are differentially regulated as the cAMP-dependent protein kinase (PKA) specifically regulates the assembly of the former in *S. cerevisiae*. In addition, PBs form prior to stationary phase arrest and are important for the long-term survival of quiescent cells. In contrast, SGs are not assembled until after cells have entered stationary phase and thus may serve as an important marker for this quiescent state. These evolutionarily conserved ribonucleoprotein (RNP) structures may provide the cell with a dynamic mode of compartmentalization within the eukaryotic cytoplasm. To further understand the regulation and function of these RNP assemblies, we examined the localization of the entire set of *S. cerevisiae* protein kinases and phosphatases during the entry into stationary phase. Interestingly, ~20% of these enzymes were found to relocate to discrete cytoplasmic foci. The first three groups of localization patterns include enzymes that localize to either PBs or SGs specifically and those found at both types of granules in stationary phase cells. As with the core constituents, the localization to PBs was regulated by PKA activity and was dependent upon the presence of a key scaffolding protein, Pat1. Finally other enzymes were recruited to either actin bodies or the phagophore assembly site or novel foci not coincident with other known structures. Importantly, all of the foci tested were found to disassemble rapidly upon the exit from stationary phase and the constituent proteins quickly re-established their original localization patterns. Our future efforts aim to determine the mechanistic basis and the physiological reasons for sequestering these enzymes into discrete sites in the cytoplasm. The latter possibilities include the removal of deleterious activities from the cytoplasm or the storage of those proteins needed for the subsequent resumption of growth. This understanding should provide important insights into the physiological role of these RNP granules in the long-term survival of quiescent cells.

6. Novel pathways of transcription regulation during the transition from growth to quiescence. *Shawna Miles, Amali P. Abeysinghe,* ***Linda L. Breeden***. Basic Sci Div, Fred Hutchinson Cancer Res Ctr, Seattle, WA.
Complex organisms depend on quiescent cells for their controlled growth, development and tissue renewal. Unscheduled exit or failure to enter this quiescent state results in uncontrolled proliferation and cancer. Yeast cells also enter a stable, protected and reversible quiescent state. As with higher cells, they exit the cell cycle from G1, reduce growth, conserve and recycle cellular contents. These similarities, and the fact that the mechanisms that start and stop the cell cycle are fundamentally conserved lead us to think that understanding how yeast enter, maintain and reverse quiescence could provide important new insights into the conserved steps in this process. Yeast that naturally exhaust the glucose from their medium differentiate into three distinct cell types that are distinguishable by flow cytometry. Among these three cell types is a quiescent population, which is so named because of its readily reversed G1 arrest, heat tolerance and longevity. The transition to quiescence involves highly asymmetrical cell divisions, cell wall fortification and G1 arrest. We find that G1 arrest is initiated well before all the glucose has been scavenged from the media, and it is maintained by the transcriptional repression of the G1 cyclin, CLN3, by Xbp1. Xbp1 is induced as glucose is depleted and it is among the most abundant transcripts in quiescent cells. Xbp1 represses 15% of all yeast genes, and failure to repress some or all of these targets leads xbp1 cells to enter a permanent arrest or senescence with a shortened lifespan. The Rad53-mediated replication stress checkpoint reinforces the arrest and becomes essential when Cln3 is overproduced. Cln3-dependent kinase activity drives the G1 to S transition by activating the transcription factor complexes SBF and MBF. We will show that two proteins associated with SBF and MBF are also required for the G1 arrest that promotes quiescence. These two related proteins, Msa1 and Msa2, have modest phenotypes during logarithmic growth, but they play critical overlapping roles in achieving both the G1 arrest and the growth arrest that are required to maintain viability as cells enter the quiescent state. Efforts to define the mode of action and the critical targets of these transcription factors will be described.

7. The inferred stress-activated signaling network from yeast: coordination, interconnectivity, and a novel NaCl network hub, Cdc14 phosphatase. ***Yi-Hsuan Ho***[1,2], *Deborah Chasman*[3], *Matthew MacGilvray*[1], *James Hose*[1], *Anna Merrill*[4], *Joshua Coon*[4,5], *Mark Craven*[3,5], *Audrey Gasch*[1,2,4]. 1) Laboratory of Genetics; 2) Cellular and Molecular Biology; 3) Department of Computer Sciences; 4) Department of Chemistry; 5) Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI.
Stressed cells coordinate a multi-faceted response spanning many levels of physiology. Yet knowledge of the complete stress-activated regulatory network as well as principles for signal integration remain incomplete. We developed an experimental and computational approach to integrate available protein interaction data with gene fitness contributions, mutant transcriptome profiles, and phospho-proteome changes in cells responding to salt stress, to infer the salt-responsive signaling network in yeast. The inferred subnetwork presented many novel predictions by implicating new regulators, uncovering unrecognized crosstalk between known pathways, and pointing to previously unknown hubs of signal integration. We exploited these predictions to show that Cdc14 phosphatase plays a central role in integrating the response. We found that the *cdc14-3* mutant at the non-permissive temperature had a distinct defect in NaCl transcriptome changes that significantly overlapped with targets of the Hog1 kinase. Based on the predictions of the network, we showed that Cdc14 is required for normal nuclear localization of Hog1 upon salt stress. We also found that Cdc14 interacts with another implicated regulator, the CK2 kinase, which together with Hog1 is required for normal induction of salt-responsive targets of the regulator Hot1. Surprisingly, we discovered that the *cdc14-3* mutant aberrantly induced G1- and S-phase genes upon NaCl treatment, even though the cells were arrested in M-phase, suggesting that it is required to suppress signaling to the cell cycle network. The network predicted that this crosstalk was mediated by the Snf1 kinase. We found that the aberrant induction was abrogated with *snf1* deletion in the *cdc14-3* background. Together, our results demonstrate the central role of Cdc14 in coordinating cellular signaling upon osmotic shock, while showcasing the predictive power of our inferred subnetwork.

8. An integrated 'omics approach to large-scale quantitative analysis of cellular metabolic regulation. ***Sean Hackett***, *Vito Zanotelli, David Perlman, Joshua Rabinowitz.* Department of Chemistry and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.
The aim of the present work is to develop a quantitative, genome-scale approach to elucidation of metabolic regulation in S. cerevisiae. While previous attempts at inferring reaction mechanisms have had limited success for all but the smallest networks, we use a novel and scalable strategy that harnesses integrative omics to infer the kinetic form of reactions. This entails finding regulators and kinetic parameters that result in the greatest consistency between flux implied by metabolite and enzyme concentrations and measured flux. Experimentally, we grew yeast in chemostats at 25 different metabolic steady states. Concentrations of metabolites and enzymes were measured by LC-MS-based metabolomics and LC-MS/MS-based proteomics. To infer fluxes, we measured uptake and excretion rates of metabolites and detailed biomass composition; together these measurements were sufficient to constrain a genome-scale flux-balanced metabolic model, resulting in reliable determination of many core metabolic fluxes. We obtained flux, enzyme concentration and all relevant metabolite concentrations across all 25 conditions for ~ 75 reactions. For about one third of the ~ 75 reactions, the concentrations of the enzyme, substrates and products were consistent with fluxes carried using Michaelis-Menten kinetics. For the majority of reactions, regulation by allostery was strongly supported. This allowed us both to reproduce canonical allosteric regulation, such as in amino acid biosynthesis, and to identify novel regulation. Beyond providing proof of concept for integrative omic analysis of metabolic regulation, this work addresses some larger questions, e.g., how much of metabolic flux control resides in enzyme concentrations versus metabolite concentrations. Our results show that across the tested physiological steady states, enzyme and metabolite concentrations make nearly equal flux-control contributions. Analyzing many metabolic steady states via integrative omics thus may address the nature of metabolic regulation and identify specific physiologically-relevant instances of regulation.

9. The calcineurin signaling network evolves via conserved kinase-phosphatase modules that transcend substrate identity. ***Jagoree Roy***[1], *Aaron Goldman*[1], *Bernd Bodenmiller*[2], *Stefanie Wanka*[2], *Christian Landry*[3], *Ruedi Aebersold*[4,5], *Martha Cyert*[1]. 1) Department of Biology, Stanford University, Stanford, CA; 2) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland; 3) Institut de Biologie Intégrative et des Systèmes, Département de Biologie, Université Laval, Québec, Canada; 4) Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, Zürich, Switzerland; 5) Faculty of Science, University of Zürich, Zürich, Switzerland.
To define the first functional network for calcineurin, the conserved Ca2+/calmodulin-regulated phosphatase, we systematically identified its substrates in S. cerevisiae using phosphoproteomics and bioinformatics, followed by co-purification and dephosphorylation assays. Substrate identification for phosphatases is particularly challenging due to the requirement for prior relevant phosphorylation. We devised a novel method to overcome these obstacles in order to robustly identify calcineurin substrates. This is the first global analysis that delineates substrates of a phosphatase. This study establishes new calcineurin functions and further elucidates the roles for calcium/calcineurin signaling in various biological processes such as the pheromone induced mating pathway, alkaline stress response and cell cycle regulation.

Further, our study reveals mechanisms that shape calcineurin network evolution. Analyses of closely related yeasts show that many proteins were recently recruited to the network by acquiring a calcineurin-recognition motif. Calcineurin substrates in yeast and mammals are distinct due to network rewiring but, surprisingly, are phosphorylated by similar kinases. We postulate that co-recognition of conserved substrate features, including phosphorylation and docking motifs, preserves calcineurin-kinase opposition during evolution. One example we document is a composite docking site that confers substrate recognition by both calcineurin and MAPK. We propose that conserved kinase-phosphatase pairs define the architecture of signaling networks and allow other connections between kinases and phosphatases to develop and establish common regulatory motifs in signaling networks.

10. Heritable capture of heterochromatin dynamics in *Saccharomyces cerevisiae*. ***Anne Dodson****, Ryan Janke, Kathryn Sieverman, Jasper Rine.* Dept of Molecular and Cell Biology, UC Berkeley, Berkeley, CA.
Gene repression of *HML* and *HMR* by the SIR proteins is sufficiently strong such that previous efforts to detect transcription of these loci have been pushed to the limit of detection without success. Meanwhile, the role of Sir1 in establishing but not maintaining silencing remains paradoxical under the assumption that wild-type cells never lose silencing. To raise the sensitivity of detection to new levels, we placed the *cre* recombinase gene under the control of silenced promoters at *HML* and *HMR* in cells in which expression of *cre* causes a permanent and heritable switch from expressing RFP to GFP, thus allowing the potential to trap transient losses of silencing. This innovation has revealed that temporary lapses in silencing occur at *HML* and *HMR*, opening the door to a wide array of new studies at the single-cell level. We quantified the rate of spontaneous losses of silencing, and have discovered that some loss events reflect local changes at *HML* or *HMR* whereas others reflect more global perturbations. Live cell imaging has allowed us to visualize the lineages and cell-cycle patterns of loss-of-silencing events. Using single-molecule RNA FISH, we have shown that even a few *cre* transcripts per cell are sufficient to allow the recombination. Moreover, RNA FISH has allowed us to establish that the two heritable expression states of *sir1* mutants reflect full repression or full expression of *HML* and *HMR*. The sensitivity of the Cre-based assay has revealed multiple roles of chromatin-modifying proteins in silencing that were previously undetectable. We have also tested whether the heterochromatic state at *HML* or *HMR* can persist through the molecular invasions required for mating type inter-conversion and find evidence for transient losses of silencing during some double-strand break repairs. Furthermore, we found that changes in yeast metabolism, including growth on different carbon sources, impact the stability and inheritance of the silent state. In addition, a mutation in an isocitrate dehydrogenase gene that causes production of the onco-metabolite 2-hydroxy-glutarate controls the stability of heterochromatin and the level of histone H3 methylation. Finally, we have adapted this approach to comparative studies of promoter-specific repression of the Galactose regulon, and found that the stability of *GAL1* repression by glucose differs between microenvironments within a single colony.

11. Dissecting the crosstalk between histone H2B ubiquitination and histone H3 methylation. *Hanneke Vlaming[1], Tibor van Welsem[1], Erik de Graaf[2], Maarten Altelaar[2], David Ontoso[3], Pedro San-Segundo[3],* ***Fred van Leeuwen***[1]. 1) Div Gene Regulation, Netherlands Cancer Inst, Amsterdam, The Netherlands; 2) Biomolecular Mass Spectrometry and Proteomics, Univ of Utrecht, The Netherlands; 3) Instituto de Biología Funcional y Genómica, Univ of Salamanca, Spain.
Post-translational modifications on histone proteins are key regulators of chromatin structure and function. Histone H2B ubiquitination is a dynamic modification that promotes the methylation of histone H3 on lysine 79 (H3K79) and lysine 4 (H3K4). This crosstalk is important for the DNA damage response and aberrant placement of all three marks has been implicated in cancer. To learn which molecular features of ubiquitinated H2B are important for the trans-histone crosstalk in vivo, we engineered yeast strains with ubiquitins tethered to every nucleosome. These ubiquitins promoted H3K79 and H3K4 methylation, both from in the vicinity of the native ubiquitination site and from a more distal site. This plasticity indicates that several features of ubiquitinated H2B are not critical for the crosstalk: the exact location of the attachment site, the native ubiquitin-lysine linkage and ubiquitination cycles. Ubiquitin tethered in the opposite orientation conferred only very modest crosstalk, suggesting that the correct orientation of ubiquitin is an important structural feature for effective crosstalk. The remarkable flexibility in this trans-histone crosstalk in vivo opens up the possibility that other ubiquitination events also promote H3 methylation.

12. Identification of a nucleosome patch required for conserved histone modifications. ***Christine Cucinotta****, Alexandria Young, Karen Arndt.* Biological Sciences, University of Pittsburgh, Pittsburgh, PA.
Cells can direct gene expression by altering the chromatin landscape via histone posttranslational modifications. A prominent and conserved histone modification involved in gene expression is the monoubiquitylation of histone H2B on lysine 123 (H2B K123ub). H2B K123ub is a prerequisite for methylation of H3 lysine 4 and H3 lysine 79, both of which are associated with active transcription. Defects in these modifications result in a range of human diseases. While the enzymes that promote these modifications are known, regions of the nucleosome required for recruitment of these enzymes are undefined. To identify histone residues required for H2B K123ub, we exploited a known genetic interaction between the E3 ubiquitin ligase, *RKR1*, and H2B K123ub in *Saccharomyces cerevisiae*. We performed a synthetic lethal

screen with cells lacking *RKR1* and a comprehensive library of H2A and H2B residue substitutions. We found novel H2A residues that are required for H2B K123ub, and they largely map to the nucleosome acidic patch. Mutations in this nucleosome patch confer varying histone modification defects downstream of H2B K123ub, indicating this region contributes differentially to histone modifications. Interestingly, mutations in this patch result in aberrant transcription. Furthermore, we found that this patch is required for recruitment of H2B K123ub machinery to active genes. Together, our findings describe a novel role for the nucleosome acidic patch in recruitment of histone modification machinery and maintenance of transcriptional integrity.

13. Mitochondrial feedback control through global H3K4 demethylation. ***Maria Soloveychik***[1], *Mengshu Xu*[1], *Ashrut Narula*[1], *Adam Rosebrock*[2], *Amy Caudy*[2], *Marc Meneghini*[1]. 1) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Donnelly Centre for Cellular and Biomolecular Research and Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.
The deposition and removal of histone modifications occurs through biochemical processes dependent on varied metabolites, supporting the emerging view that chromatin and metabolism are linked. Although this phenomenon is well documented in the case of histone acetylation and de-acetylation, whether normal metabolism impacts histone demethylation, and whatever physiological consequences such an impact might have, remains unknown. Enzymatic activity of Jumonji-family histone demethylases requires alpha-ketoglutarate (KG), a metabolite produced during tricarboxylic acid cycle (TCA) flux and glutamate catabolism. Using nutritional manipulation of these pathways in the budding yeast Saccharomyces cerevisiae, we modulated levels of cellular KG. Here we show that Jhd2, a JARID1-family histone H3 lysine-4 (H3K4) demethylase, accomplished global demethylation of H3K4 under conditions of elevated KG. Transcript profiling under these conditions showed that Jhd2 repressed the accumulation of mRNAs encoding mitochondrial proteins and promoted the accumulation of mRNAs encoding ribosomal and glycolytic proteins. Indeed, a prominent number of JHD2-repressed genes impact the electron transport chain (ETC), a conserved system that couples TCA produced NADH to energy production and oxygen consumption through respiration. Cell-biological studies confirmed that JHD2 repressed mitochondrial respiration through H3K4 demethylation. In accordance with this, JHD2 also promoted fermentative metabolism, with JHD2-overexpressing cells producing higher amounts of ethanol and *jhd2* cells producing less. The metabolic regulatory function we have identified for JHD2 impacts yeast metabolism in a way that that is analogous to Warburg metabolism, a hyper-glycolytic/hypo-respiratory mode of glucose utilization that is ubiquitous to cancerous and other proliferative cells in humans. In accordance with widespread evidence associating mitochondrial function with lifespan control in varied organisms, we found that JHD2 was necessary and sufficient to limit yeast replicative lifespan. These findings demonstrate that cellular metabolic state can impact histone demethylation through regulated activity of Jumonji-family proteins. Specifically, In yeast, Jhd2 demethylation of H3K4 occurred under conditions of elevated KG. Interestingly, the output of this pathway impacts Warburg metabolism and lifespan, suggesting a physiological homeostatic mechanism controlled through H3K4 demethylation.

14. Role of ATP-dependent chromatin remodeling enzyme Fun30 in co-transcriptional pre-mRNA splicing. *Qiankun Niu, Wei Wang, Boseon Byeon, Yong Li, Asim Bikas Das,* ***Wei-Hua Wu***. Neuroscience and Regenerative Medicine, Medical College of Georgia, Georgia Regents University, Augusta, GA.
The primary structure of eukaryotic chromatin, manifested through nucleosome positioning and occupancy, has been linked to the regulation of co-transcriptional pre-mRNA splicing. Studies have revealed that nucleosomes are preferentially enriched at exons, flanked by relatively nucleosome-free regions of introns. Likewise, histone modifications including trimethylated lysine 4 residue of histone H3 (H3K4me3), H3K36me3 and H2BK123 ubiquitination (H2BK123ub) are enriched specifically in exons. These observations suggest a role of chromatin in exon recognition for accurate and efficient pre-mRNA splicing. However, the underlying mechanism for the differential distribution of nucleosome occupancy and histone modifications in exons relative to introns is not known. The evolutionarily conserved ATP-dependent chromatin remodeling enzyme Fun30 has been shown previously to regulate heterochromatin silencing, transcription and DNA resection during homologous recombination. In this study, we identified a novel function of Fun30 in regulation of co-transcriptional pre-mRNA splicing. We found that the level of pre-mRNAs was accumulated in Fun30-depleted cells. Analyzing published ChIP-seq data revealed that Fun30 was enriched in the gene body of intron-containing genes, near exon-intron junctions. Furthermore, the recruitment of spliceosome components was reduced at individual intron-containing genes in Fun30-depleted cells. These results suggest that Fun30 plays a role in promoting pre-mRNA splicing. Given that majority of splicing events occur co-transcriptionally and that RNA polymerase II (RNAPII) pause is important for the recruitment of spliceosome, we examined RNAPII enrichment in the wild type and fun30 strains by ChIP. We found that, while the level of total RNAPII remained stable, the enrichment profile of CTD Ser2 phosphorylation was significantly changed in intron-containing genes in the fun30 mutant. In addition, we found that Fun30 interacted with the PAF1 complex, which has been previously implicated in regulation of RNAPII elongation rate and H3K36me3 and H2BK123ub modifications. Taken together, these results suggest that the ATP-dependent chromatin

remodeling enzyme Fun30 promotes co-transcriptional pre-mRNA splicing by regulating the ratio of RNAPII CTD Ser5 and Ser2 phosphorylation at intron-containing genes.

15. Gene Loops Facilitate mRNA Export in Yeast. *Badri Nath Singh, **Michael Hampsey**.* Dept Biochem and Mol Biol, Rutgers - R W Johnson Med Sch, Piscataway, NJ.
Extraordinary progress has been made in recent years toward understanding the structure of the eukaryotic genome and the mechanisms that underlie gene expression. Missing from the picture, though, is a fundamental understanding of the three-dimensional organization of the genome and how these structures affect gene expression. We are investigating the higher order structure of genes transcribed by RNA polymerase II (RNAP II) in the yeast Saccharomyces cerevisiae. Using a powerful technique, called chromosome conformation capture (CCC), designed to probe the three-dimensional architecture of the genome, we have detected gene loops that specifically juxtapose promoter and terminator regions. Gene loops are formed on actively transcribed genes and are dependent upon components of both the initiation and pre-mRNA 3-end processing machineries. However, neither the structural requirements for the formation/maintenance of gene loops, nor the functions of gene loops are well understood. Here we report that gene loops are RNA-dependent and facilitate export of mRNA from the nucleus to the cytoplasm. Furthermore, our systematic analysis of genetic and physical requirements for looping (i) define the transcription and mRNA export factors TREX-1 and TREX-2 as looping requirements; and (2) determine the function of gene loops by defining how gene loops, the nuclear pore complex (NPC), and mRNA export are interconnected. Our working hypothesis is that all protein-encoding genes form loops. Looping is not essential for gene expression, but rather facilitates mRNA export by interaction with the NPC. This is consistent with (i) coupling of export to transcription of nascent mRNA and (ii) migration of actively transcribed genes to the NPC. Our results define a fundamental process involved in gene expression and illuminate the pathway by which transcription is coupled to mRNA export.

16. The budding yeast polo kinase, Cdc5, is a regulator of nuclear morphology. ***Alison D. Walters***[1]*, Christopher K. May*[1]*, Emma Dauster*[1]*, Bertrand P. Cinquin*[2]*, Elizabeth A. Smith*[2]*, Carolyn A. Larabell*[2]*, Orna Cohen-Fix*[1]. 1) Laboratory of Cell and Molecular Biology, NIDDK/NIH, Bethesda, MD; 2) Dept of Anatomy, School of Medicine, UCSF, San Francisco, CA.
The nuclei of cells of a given cell type have a particular size and shape. Abnormalities in nuclear size and shape are observed in several diseases states. Despite these long-standing observations, our understanding of the factors determining nuclear size and shape is poor. In higher eukaryotes, the nuclear envelope (NE) must expand after it is reformed at the end of mitosis to allow for chromosome decondensation. In yeast, which undergo closed mitosis, the NE must expand to allow chromosome segregation and the formation of two daughter nuclei. The mechanisms by which NE expansion is achieved and controlled are unknown. Work in our lab has shown that when *S. cerevisiae* is delayed in mitosis the NE continues to expand despite the fact that chromosome segregation is paused, and a nuclear extension, or flare, is formed adjacent to the site of the nucleolus. To identify genes involved in NE expansion, we carried out a screen for mutants that do not form a flare when arrested in mitosis. We identified a point mutation in the yeast polo kinase, CDC5, which gave rise to a highly penetrant no-flare phenotype during a mitotic arrest. We found that Cdc5 is required for both flare formation and flare maintenance, confirming that its effect on nuclear morphology is both active and reversible in mitosis. We have further shown that Cdc5 not acting through the well-characterized FEAR/MEN pathways to affect nuclear morphology. We used soft X-ray tomography to measure nuclear surface area and nuclear volume as WT and *cdc5-nf* cells entered a mitotic arrest. Our data suggest that *cdc5-nf* mutants expand their nuclei isometrically rather than forming a flare. This observation indicates that Cdc5 plays a role in confining NE expansion to the nucleolar region during a mitotic arrest. We went on to observe that small NE expansions occur at the nucleolus prior to anaphase in an uninterrupted cell cycle and that these mini-flares are also Cdc5 dependent. Our data provides the first evidence that Cdc5, a key regulator of mitosis, plays a role in regulating nuclear morphology and NE expansion.

17. Surveying the Inner Nuclear Membrane Landscape. ***Christine J Smoyer**, Jennifer Gardner, Sreenivasulu Santharam Katta, Brian Slaughter, Jay Unruh, Dan Bradford, Scott McCroskey, Sue Jaspersen.* Stowers Institute for Medical Research, Kansas City, MO. 64110.
The eukaryotic cell nucleus contains genetic material as well as proteins involved in gene expression, DNA replication and repair, chromosome segregation and other processes essential for genomic integrity and cell proliferation. The nuclear membrane is a double lipid bilayer. The outer nuclear membrane (ONM) is contiguous with the endoplasmic reticulum (ER) and shares various integral membrane components. Proteomic analysis of the inner nuclear membrane (INM) indicates that it is composed of at least 100 distinct proteins, many of which are uncharacterized. However, proteomic approaches are based on a subtractive method meaning that proteins with dual roles in the INM and ER are likely overlooked. Proteins of low abundance and/or solubility are also unlikely to be discovered in a biochemical approach, thus, we have turned to a cell biological strategy to understand INM composition. Using a variation of the bimolecular fluorescence complementation assay, we can detect proteins that localize specifically to the INM using live cell imaging.

We have systematically analyzed approximately 850 membrane (or membrane predicted) proteins in our screen for INM localization. Among the hits that we have uncovered using this approach are three components involved in remodeling of the nuclear membrane: Brr6, Brl1 and Apq12. Interestingly, the genes encoding these proteins are present only in organisms that undergo a closed mitosis, suggesting that they may play a role in nuclear envelope insertion of the spindle pole body (SPB). To test this idea, we generated alleles of each gene and screened for temperature-sensitive mutants. Further analysis of one allele of *BRR6* revealed a novel role in SPB separation and bipolar spindle formation as well as dramatic effects on nuclear membrane morphology and export of molecules from the nucleus. The identical mutation in the paralog, *BRL1*, had no observable phenotype, supporting the idea that the function of these genes has diverged. We are currently investigating how Brr6 contributes to the processes of nuclear pore complex and SPB insertion through its role in nuclear membrane integrity by identifying Brr6 binding partners.

18. SNAREs and the Dsl1 tethering complex mediate an alternative, Sey1p-independent ER fusion pathway. ***Jason V. Rogers***[1], *Conor McMahon*[1], *Anastasia Baryshnikova*[2,3,4], *Michael Costanzo*[2], *Charles M. Boone*[2,3], *Frederick M. Hughson*[1], *Mark D. Rose*[1]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, M5S 3E1, Canada; 3) Department of Molecular Genetics, University of Toronto, Toronto, M5S 1A8, Canada; 4) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544.
The endoplasmic reticulum (ER) in yeast forms an organized network of tubules and sheets at the cell cortex that is continuous with the outer nuclear envelope. The characteristic lattice shape is created and maintained by ER-ER fusion. The dynamin-like GTPase Sey1p has been implicated as the primary ER fusogen, but sey1 cells do not have a growth or ER structure defect. Recent work by our group and others demonstrated strong synthetic growth and ER structure defects in cells containing both a sey1 deletion and a mutation in any of the ER-associated SNAREs responsible for retrograde vesicle trafficking. These data suggest that SNAREs mediate an alternative, Sey1p-independent ER fusion pathway. However, it remained possible that the synthetic defects are due to downstream effects of perturbed retrograde vesicle trafficking. Moreover, it remained unclear whether the SNAREs act in isolation, or in combination with their normal vesicle trafficking accessory proteins, including the Dsl1 tethering complex and the COPI coat complex. We used candidate and synthetic genetic array (SGA) approaches to characterize and identify additional components of the SNARE-mediated ER fusion pathway. We found that sey1 cells combined with mutations in the Dsl1 tethering complex have strong synthetic growth, ER structure, and ER fusion defects. Importantly, sey1 cells combined with mutations in the COPI vesicle coat (which is essential for normal retrograde trafficking) do not have synthetic growth or ER structure defects. Therefore, retrograde trafficking defects do not generally cause synthetic ER structure and growth defects. We conclude that the SNAREs and the Dsl1 tethering complex directly mediate a Sey1p-independent ER fusion pathway. Finally, we found that sey1 dsl1 yop1 rtn1 cells are extremely slow-growing and almost inviable. Yop1p and Rtn1p are the two primary reticulons, which are responsible for forming ER tubules and maintaining their structure after ER fusion. This is the first demonstration that ER structure and fusion are essential processes in yeast, and suggests that redundancy between the reticulons and the two ER fusion pathways completely explains ER structure and consequent cell viability.

19. Plasma membrane localized KDEL receptors ensure endocytic entry and retrograde transport of a viral A/B toxin in yeast. ***B. Becker***, *A. Blum, D. Rammo, M.J. Schmitt.* Molecular and Cell Biology, Saarland University, Saarbrücken, Saarbrücken, Germany.
A/B toxins such as Cholera toxin and the yeast K28 toxin possess an H/KDEL amino acid motif at the C-terminus of their cell binding B-subunits which is recognized and bound by KDEL receptors (KDELRs) of their target cells. The main function of KDELRs is to retrieve resident ER proteins that escaped from the secretory pathway back to the ER. Until now, it was believed that the initial toxin interaction with KDELRs occurs within the Golgi, i.e. after receptor-mediated endocytosis and endosomal trafficking. However, we recently demonstrated that the yeast KDEL receptor Erd2p is not only present in the ER and Golgi but also in the plasma membrane (PM). We demonstrate that PM localized Erd2p is capable to bind Kar2p/BiP from outside the cell, leading to its endocytosis and retrograde transport to the ER where it restores the growth defect of a kar2ts mutant. In addition to its essential physiological role in the uptake of secreted HDEL-bearing proteins from the PM, we show that Erd2p is crucial for A/B toxin binding and endocytosis. Consequently, we used K28 as model cargo to mechanistically dissect the internalization of A/B toxins and to understand the general basis of receptor endocytosis in yeast and higher eukaryotic cells. In vivo topology of Erd2p matches the postulated topology of KDELRs in mammalian and plant cells. Interestingly, three potential endocytic motifs within each KDELR ensure recognition and internalization of HDEL-cargo from the PM. By using biochemical analysis, rational receptor mutagenesis and toxin sensitivity assays, toxin/receptor internalization was shown to be regulated by multiple mechanisms, amongst which (i) (mono)ubiquitylation of Erd2p via Ubc4p (E2) and Rsp5p (E3), (ii) the involvement of AP-2 complex components as well as (iii) the Sla1p/Pan1p complex were identified as being key elements in KDELR endocytosis.

20. Elucidating the architecture and function of the yeast exocyst complex. ***Mary Munson***[1], *Margaret Heider*[1], *Caroline Duffy*[1], *Zhanna Hakhverdyan*[2], *Raghav Kalia*[3], *Nicholas Farrall*[3], *Michael Rout*[2], *Adam Frost*[3]. 1) Biochemistry & Molecular Pharmacology, UMass Medical School, Worcester, MA; 2) Laboratory of Cellular and Structural Biology, The Rockefeller University, NY; 3) Department of Biochemistry, University of Utah, Salt Lake City, UT.
Eukaryotic cells are crowded with membrane-bound vesicles that transport cargo between subcellular organelles, and to the plasma membrane for secretion. Highly conserved machinery has evolved for spatial and temporal control of this multitude of membrane fusion events, but the molecular details of these processes are not well understood. SNARE proteins are core components of the membrane fusion machinery, and regulation of the SNAREs is crucial for the precise specificity and timing of fusion. Several protein families are critical for SNARE regulation, including large multisubunit tethering complexes, such as the exocyst complex at the plasma membrane. The exocyst is essential for cellular growth, secretion and endocytosis. It is a hetero-octameric protein complex localized to sites of secretion on the plasma membrane, where it is thought to function in quality control through specific tethering of secretory vesicles. Our earlier genetic, biochemical and phenotypic studies indicate functional cooperation between the yeast exocyst complex and the SNARE regulatory protein Sec1 to regulate the specificity and timing of SNARE complex assembly and membrane fusion. We also identified conserved residues on the surface of the exocyst subunit Sec6 that are required for localizing the exocyst to sites of secretion. Further studies of the exocyst architecture and interactions with binding partners, including the SNAREs, Sec1 and the Rab-GTPase Sec4, however, were previously hindered by the challenges of obtaining purified complexes. We recently developed a successful purification strategy for obtaining native, intact yeast exocyst complexes, and are investigating their structure and stoichiometry using several approaches, including biochemical, genetic and proteolytic disruptions of the complex, mass spectrometry, chemical crosslinking, and binding studies with recombinant partners. By altering solution conditions, we could also purify partially disassembled exocyst, which revealed novel subcomplexes that are likely to be important for assembly and function. Moreover, negative stain electron microscopy revealed our first view of the overall structure of the intact yeast exocyst complex.

21. The molecular architecture of the Target Of Rapamycin Complex 2 reveals why it is insensitive to rapamycin. *C. Gaubitz*[1,4], *T. Maia de Olivera*[2,4], ***Manoël Prouteau***[1,4], *A. Leitner*[3], *M. Karuppasamy*[2], *D. Rispal*[1], *S. Eltschinger*[1], *G. Robinson*[1], *G. Konstantinidou*[1], *S. Thore*[1], *R. Aebersold*[3], *R. Loewith*[1,5], *C. Schaffitzel*[1,5]. 1) Molecular Biology Dept, University of Geneva, Geneva, Switzerland; 2) EMBL, Grenoble, France; 3) Institute of Molecular Systems Biology, ETHZ, Zurich, Switzerland; 4) Co-first authors; 5) Co-last authors.
Rapamycin is an antifungal produced by the soil bacterium *Streptomyces hygroscopicus*. Genetic screens performed in *Saccharomyces cerevisiae* defined the mode of action of this macrolide: in cells, rapamycin highjacks the proline isomerase FKBP12 (FK506 Binding Protein 12kDa) to form a toxic complex that then binds and inhibits the essential Targets Of Rapamycin (encoded by TOR1 and its paralog TOR2). Tor1 and Tor2 are the founding members of the phosphatidylinositol 3-kinase-related kinase (PIKK) family which are Ser/Thr protein kinases that curiously resemble PI lipid kinases. Tor1 and Tor2 play essential roles in the regulation of eukaryote growth by as components of two distinct, multiprotein complexes, TORC1, which is sensitive to rapamycin, and the lesser characterized TORC2, which is unsensitive. In yeast, TORC2 regulates tensional homeostasis of the plasma membrane, actin polarization, endocytosis, calcineurin activity and genome integrity although the molecular mechanisms these distal regulations are not known. TORC2 contains six subunits: Tor2, Lst8, Avo1, Avo2, Avo3 and Bit61 or its paralog Bit2; but the overall TORC2 organization and structure are unknown. Using integrated structural approaches combining cryo electron microscopy and isotopic crosslinking mass spectrometry, we describe the molecular architecture of TORC2. Like TORC1, TORC2 displays a rhomboid shape with C2 symmetry and a prominent central cavity. Avo2 and Bit61/2 are positioned at the periphery of the complex. Lst8 is in a close association with the middle portion of Avo1. Avo3 is wrapped around the Tor2 kinase domain and situated close to the FKBP12-rapamycin binding domain of Tor2. Truncation in Avo3 makes TORC2 sensitive to inhibition by FKBP12-rapamycin and provides a tool to study TORC2 function *in vivo*. Docking of pseudo-atomic models of TORC2 subunits into the cryo-EM reconstruction affords a framework to understand the regulation and the substrate specificity of TOR complexes.

22. Higher-order epistasis between a mutation and four or more segregating variants generates a 'new' phenotype in a cross. *Matthew Taylor*, ***Ian Ehrenreich***. University of Southern California, Los Angeles, CA.
Recent research suggests that genetic interactions involving more than two loci may influence a number of complex traits. How these higher-order interactions arise at the genetic and molecular levels remains an open question. To provide insights into this problem, we dissected a colony morphology phenotype that segregates in a yeast cross and results from synthetic higher-order interactions. Using backcrossing and selective sequencing of progeny, we found five loci that collectively produce the trait. We fine-mapped these loci to 22 genes in total and identified a single gene at each locus that caused loss of the phenotype when deleted. Complementation tests or allele replacements provided support for functional variation in these genes, and revealed that pre-existing genetic variants and a spontaneous mutation interact to cause the trait. The causal genes have diverse functions in endocytosis (END3), oxidative stress response (TRR1), RAS-cAMP

signalling (IRA2), and transcriptional regulation of multicellular growth (FLO8 and MSS11), and for the most part have not previously been shown to exhibit functional relationships. Further efforts uncovered two additional loci that together can complement the non-causal allele of END3, suggesting that multiple genotypes in the cross can specify the same phenotype. Our work sheds light on the complex genetic and molecular architecture of higher-order interactions, shows how such interactions can generate 'new' phenotypes, and raises questions about the broader contribution of such interactions to heritable trait variation.

23. A quantitative study of whether the HSP90 chaperone modulates robustness to new mutations, recombination, and standing variation in yeast. ***Kerry A. Geiler-Samerotte****, Mark L. Siegal.* Center for Genomics and Systems Biology, New York University, New York, NY.
The molecular chaperone HSP90 has been proposed to provide robustness to mutations, allowing them to accumulate in genomes while their phenotypic effects are hidden until HSP90 function is compromised. This storage and eventual release of variation is hypothesized to help organisms survive stressful conditions when the revealed variation is adaptive. Using bakers yeast, Saccharomyces cerevisiae, we quantify the degree to which HSP90 provides robustness to three different kinds of genetic perturbations: 1. new mutations before selection (using mutation accumulation lines), 2. recombination (using segregants from a mapping family) , and 3. standing genetic variation (using wild strains and strains gathered from breweries). We use high-throughput single-cell microscopy and linear modeling to quantify variance in cell morphology with and without HSP90 inhibition, surveying 220 morphological parameters such as cell size and nuclear position using Calmorph software. We find that no group of strains demonstrates increased morphological variance when HSP90 function is impaired. This result suggests that HSP90 does not confer robustness to the genetic perturbations that we study. However, inhibiting HSP90 does have some effect: it interacts epistatically with mutations, altering the average morphology of individual strains without causing an overall increase in variance across strains. These findings suggest a reinterpretation of HSP90s role in adaptation to stressful conditions, at least in yeast lineages.

24. Diversity across the *Saccharomyces* genus and the genomic tools to tap it. ***Chris Todd Hittinger***[1]*, William G. Alexander*[1]*, Drew T. Doering*[1]*, David Peris*[1]*, Kayla Sylvester*[1]*, Diego Libkind*[2]*, Paula Gonçalves*[3]*, José Paulo Sampaio*[3]. 1) Laboratory of Genetics, Genome Center of Wisconsin, Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison; 2) Laboratorio de Microbiología Aplicada y Biotecnología, Instituto de Investigaciones en Biodiversidad y Medio-ambiente, INIBIOMA (CONICET-UNComahue), Bariloche, Argentina; 3) Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal.
Although *S. cerevisiae* is one of the best understood genetic model systems, characterization of most other *Saccharomyces* species has been limited. Recent research has substantially clarified relationships between the natural species of the genus and key industrial hybrids that contain genetic contributions from *S. cerevisiae*, *S. kudriavzevii*, *S. uvarum*, and/or *S. eubayanus*. Here we use a population and phylogenomics approach to show that the two sister species, *S. eubayanus* and *S. uvarum*, can be further subdivided into four diverse but highly structured populations that are sympatric in Patagonian southern beech forests. First, we identify which population of *S. eubayanus* is the closest known relative of the progenitor of lager-brewing yeast (*S. cerevisiae x S. eubayanus*). Second, we demonstrate that one Patagonian population of *S. uvarum* is closely related to strains from across North America, Europe, and Asia that have low genetic diversity but include many strains used in the fermentation of champagne and similar products. In contrast with strains from natural settings, strains associated with these industrial fermentations frequently have undergone reticulation events involving other species, such as introgression and hybridization. To facilitate targeted and high-throughput functional investigation across the genus, we also report the development of a new selectable/counterselectable marker system for genetically manipulating prototrophs. These self-contained cassettes generate targeted double-strand breaks to efficiently engineer a target locus in both haploid and diploid strains. With general tools to precisely engineer diverse wild strains from across the genus, we anticipate a golden era of evolutionary genetics research with *Saccharomyces* as a model genus.

25. High-throughput functional screening of driver mutations. ***Celia Payen***[1]*, Anna Sunshine*[1]*, Giang Ong*[1]*, Wei Zhao*[2]*, Maitreya Dunham*[1]. 1) Genome Sciences, University of Washington, Seattle, WA; 2) Biostatistics, Universty of Washington, Seattle, WA.
High-throughput sequencing technologies have enabled us to expand the scope of genetic screens to identify mutations that underlie subtle phenotypes, such as small fitness improvements that occur during the course of experimental evolution. However, like in high-throughput sequencing of human cancers, functional tests are still required to identify the driver mutations amidst the large pool of mutations detected in evolved clones or populations. Here we combine functional genomics and whole genome sequencing to examine functional consequences of driver and passenger mutations, providing a clear description of the genetic landscape associated with proliferative growth and revealing an accurate prediction of evolutionary outcomes. To separate driver from passenger mutations detected during laboratory experimental evolution, we tracked thousands of barcoded, systematically created gain and loss of function mutations as they grew

competitively in continuous culture. We compared these results to a set of ~1600 recurrent and rare mutations acquired during hundreds of generations of growth in 11 different conditions. By doing so we determined that each population acquires up to five driver mutations over the course of experimental evolution. In addition, we found significant differences between the mutation spectra of haploid and diploid yeasts: in haploids we confirmed that loss of function mutations are the major adaptive strategy while in diploids, mutations predicted to modify gene expression level (5 upstream and intergenic) are statistically enriched. We also detected a bias of mutation where disruptive mutations are more likely to accumulate near the transcription start site. This comprehensive functional screen provides a detailed map of the genomic landscape associated with proliferative growth by identifying changes that contribute to the development of a cancer-like phenotype, and allows us to compare the actual, spontaneously derived mutational spectrum with mutagenic potential.

26. Genetic influences on translation in yeast. ***Frank W Albert***[1], *Dale Muzzey*[2,3], *Jonathan S Weissman*[2,3], *Leonid Kruglyak*[1,3,4]. 1) Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA; 2) Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA; 3) Howard Hughes Medical Institute; 4) Department of Biological Chemistry, University of California, Los Angeles, CA.
Heritable differences in gene expression between individuals are an important source of phenotypic variation. The question of how closely the effects of genetic variation on protein levels mirror those on mRNA levels remains open. Here, we addressed this question by using ribosomal footprinting to examine how genetic differences between the BY and RM strains of the yeast S. cerevisiae affect translation. Strain differences in translation were observed for hundreds of genes, more than half as many as showed genetic differences in mRNA levels. Similarly, allele specific measurements in the diploid hybrid between the two strains found roughly half as many cis-acting effects on translation as were observed for mRNA levels. In both the parents and the hybrid, strong effects on translation were rare, such that the direction of an mRNA difference was typically reflected in a concordant footprint difference. The relative importance of cis and trans acting variation on footprint levels was similar to that for mRNA levels. Across all expressed genes, there was a tendency for translation to more often reinforce than buffer mRNA differences, resulting in footprint differences with greater magnitudes than the mRNA differences. This contrasts with reports of translational buffering between two yeast species and suggests that translation may experience different evolutionary pressures within than between species. We also catalogued instances of premature translation termination and highlight problematic coding gene models in the yeast reference genome. Finally, we discuss methodological questions arising when comparing molecular traits, especially when the traits are quantified with different levels of measurement error.

27. Analysis of transcription activation distance as a polygenic trait in *Saccharomyces cerevisiae*. ***Caitlin Reavey***[1], *Mark Hickman*[2], *David Botstein*[3], *Fred Winston*[1]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Biochemistry, Rowan University, Glassboro, NJ; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.
Most aspects of eukaryotic transcription are conserved from yeast to human. However, the distance over which transcriptional activation can occur differs between *Saccharomyces cerevisiae* and metazoans. In *S. cerevisiae*, the upstream activating sequence (UAS) is generally found within 300 base pairs of the transcription start site (TSS); when the UAS is moved too far away, activation no longer occurs. In contrast, metazoan enhancers can activate from as far as 100 kilobases from the TSS. In past work, our lab identified single mutations that allow transcription activation to occur at a greater-than-normal distance from the *GAL1* UAS. These single mutations occurred in genes known to be involved in transcription and chromatin structure, with a mutation in *SIN4* giving the strongest phenotype. As the long-distance activation phenotype of the single mutants was weak, we have now studied long-distance activation as a polygenic trait, isolating strains with multiple mutations that together confer a strong phenotype. We isolated three strains, each containing multiple mutations that contribute to the strength of the long-distance activation phenotype. We have accounted for most of the heritability of the phenotype and have identified causative mutations in *MOT3*, *GRR1*, *MIT1*, *PTR3*, *YOR019W*, and *MSN2* that contribute to the long-distance activation phenotype. Our results suggest that different combinations of mutations allow activation at distances of at least 2 kilobases and that Mediator plays both positive and negative roles in the regulation of activation distance.

28. Mapping Recombination in Single Meiotic Cells Reveals that Tel1/ATM Controls the Positioning and Fate of DNA Double-Strand Breaks. ***Carol Anderson***, *Ashwini Oke, Phoebe Yam, Jennifer Fung.* UCSF, San Francisco, CA.
We are studying the events in meiosis that help ensure proper chromosome segregation and formation of viable gametes. During meiosis, pairs of homologous chromosomes undergo recombination, forming reciprocal exchanges known as crossovers. These physical links help homologs align properly on the Meiosis I spindle, ensuring correct segregation at the first meiotic division. Errors in this process are a leading cause of miscarriages, infertility, and birth defects in humans. Exchange occurs at deliberately induced double-stranded DNA breaks, but not all of these breaks become crossovers; some become non-reciprocal exchanges called noncrossovers that are not as essential for successful meiosis. A major

question is how the fate of each DNA break - whether it becomes a crossover or noncrossover - is determined. Using a combination of whole-genome mapping of recombination products and immunostaining of meiotic chromosomes, we have discovered that the important DNA damage signaling kinase and tumor suppressor, Tel1/ATM, is a key regulator of the crossover/noncrossover decision, specifically promoting repair of meiotic DNA breaks as crossovers. Since Tel1 interacts with unprocessed breaks, the crossover/noncrossover decision therefore likely occurs immediately after break formation, much earlier than previously suspected. In the course of this work we made another surprising discovery. Crossovers have long been known to display interference, i.e. regularized spacing along the chromosome. The mechanisms behind this phenomenon are unknown, but double-strand break precursors also appear to interfere with each other, and the prevailing assumption has been that crossover interference results at least partly from the regular spacing of double-strand break precursors. Surprisingly, we found evidence that exactly the opposite is true: regular spacing of breaks emerges from the process of establishing regularly spaced crossovers. Based on this finding we propose a new model that explains several previously mysterious aspects of the meiotic recombination landscape.

29. RAD51 and the DNA damage checkpoint are essential for increased chromosome mobility after DNA damage in diploid S. cerevisiae. ***Michael J. Smith***[1], *Fraulin Joseph*[1], *Marina Ermakova*[1], *Ignacio Izeddin*[2], *Vincent Recamier*[2,3], *Xavier Darzacq*[2], *Judith Mine-Hattab*[1,2,4], *Rodney Rothstein*[1]. 1) Department of Genetics & Development, Columbia University Medical Center, New York, NY, USA; 2) Institut de Biologie de l'École Normale Supérieure, Paris, France; 3) Laboratory Imaging, s. r. o., Prague,Czech Republic; 4) Institut Curie, Paris, France.
Homologous recombination (HR) is an essential repair pathway for resolving DNA double-strand breaks (DSBs). HR is the process by which a damaged chromosome seeks out homologous sequence in the genome and uses it as a template for repair. While many of the steps of this important pathway are well understood, the means by which the damaged chromosome locates and interacts with its undamaged homolog, a process known as homology search, are still unknown. Using high-resolution, time-lapse microscopy in living, diploid yeast cells, we have been able to track chromosomal loci through a system of fluorescent tags. We and others have previously reported that homologous chromosomes occupy different nuclear positions and are confined to a small space in the absence of damage. Following the induction of DSBs close to the tracked locus, their area of exploration greatly increases. Interestingly, we also find that uncut chromosomal loci increase in mobility in response to damage on other chromosomes, indicating a global response that is not limited to the local increase in mobility on the cut chromosome. Both of these responses are sensitive to deletion of the recombinase and RecA homolog, *RAD51*. Activation of the DNA damage checkpoint is both necessary and sufficient for this global increase in mobility, even in the absence of DNA damage. Deletion of *RAD51*, however, prevents an increase in mobility following artificial checkpoint activation, indicating a key role for Rad51 at the intersection of the local and global response to DSBs. The global increase in mobility is present throughout the cell cycle, and increased mobility in G1 also depends upon *RAD51*, even though Rad51 is not thought to form filaments on single-stranded DNA in G1. Lastly, we find that *RAD52*, which is responsible for allowing Rad51 access to single-stranded overhangs following resection, may be essential for local, but not global increases in mobility. These experiments suggest that while Rad51 filaments at breaks may be necessary for local increases in mobility, the presence of Rad51 in the nucleus is sufficient to permit increased mobility. Taken together, our results suggest a role for Rad51 in regulating chromosomal mobility that is not dependent upon filament formation at 3' ends, and point to a role for the checkpoint in initiating global increases in mobility.

30. A combined genetic and biochemical analysis of yeast telomerase. ***Johnathan W. Lubin***[1,2], *Timothy M. Tucey*[1,2], *Lisa Nguyen*[1,2], *Vicki Lundblad*[1]. 1) Salk Institute for Biological Studies, La Jolla, CA; 2) University of California, San Diego, La Jolla, CA.
Telomerase from budding yeast consists of the catalytic Est2 protein and two regulatory subunits (Est1 and Est3) in association with the TLC1 RNA, with each of the four subunits essential for in vivo telomerase function. Telomerase is highly regulated, in that only a subset of telomeres are elongated in each cell cycle; however, the mechanism(s) that restrict telomerase activity in vivo are still poorly understood. This deficit may stem from the fact that the surface of yeast telomerase represents a largely unexplored territory; specifically, we propose that there are as-yet-unidentified interaction surfaces on the three Est proteins that regulate telomerase function. To address this, we have developed a mutagenesis protocol designed to identify the rare sub-class of mutations that target functionally important residues on the surface of a protein. We initially applied this protocol to the small Est3 subunit as a proof-of-principle test (Lubin et al., 2013), which was validated once the structure of the Est3 protein was successfully determined (Rao et al. 2014). Specifically, all of the predicted surface residues identified by our genetic protocol were in fact located on the experimentally determined Est3 protein surface. These residues define two adjacent, functionally distinct patches: a narrow extended interface mediates association with telomerase, whereas an adjacent surface is utilized for a second novel function distinct from telomerase binding. We have subsequently applied this mutagenesis strategy to the Est1 and Est2 telomerase subunits, which has generated a highly curated collection of separation-of-function mutations that span both proteins. Biochemical analysis has identified regions of each protein that are required for binding to known interaction partners. For example, we have identified sites on both Est1 and Est2 that mediate association of the Est3 telomerase subunit with the complex, as well as

a novel ~90 amino acid domain in the N-terminus of the Est1 protein that mediates RNA binding. By process of elimination, we now have three clusters of residues with no known binding partners and thus define novel functions for telomerase; the results of on-going efforts to identify factors that interact with these three novel surfaces will be presented.

31. The FACT complex interacts with the E3 ubiquitin ligase Psh1 to prevent ectopic localization of CENP-A. ***Gary M Deyter**, Sue Biggins.* Basic Sciences Division, Fred Hutchinson Center, Seattle, WA.
Centromere identity and its epigenetic maintenance require the incorporation of a histone H3 variant called CENP-A at centromeres. CENP-A mislocalization to ectopic sites may disrupt chromatin-based processes and chromosome segregation, so it is important to uncover the mechanisms by which this variant is exclusively localized to centromeres. Here, we identify a role for the conserved chromatin modifying complex FACT in preventing CENP-A^{Cse4} mislocalization to euchromatin by mediating its proteolysis. The budding yeast Spt16 subunit of the FACT complex binds to Psh1, an E3 ubiquitin ligase that targets CENP-A^{Cse4} for degradation. The interaction between Psh1 and Spt16 is critical for both CENP-A^{Cse4} ubiquitylation and its exclusion from euchromatin. We found that Psh1 cannot efficiently ubiquitylate CENP-A^{Cse4} nucleosomes *in vitro*, suggesting that additional factors must facilitate CENP-A^{Cse4} removal from chromatin *in vivo*. Consistent with this, FACT releases CENP-A^{Cse4} from nucleosomes and potentiates the interaction between CENP-A^{Cse4} and Psh1 *in vivo*. Together, our data identify a previously unknown mechanism to maintain centromere identity and genomic stability through the FACT-mediated degradation of ectopically localized CENP-A^{Cse4}.

32. Reconstitution of strong kinetochore attachments requires more than the microtubule binding components of the kinetochore. ***Emily M. Mazanka**[1], Neil T. Umbreit[1], Alex Zelter[1], Daniel R. Gestaut[3], Charles L. Asbury[2], Trisha N. Davis[1]*. 1) Biochemistry, University of Washington, Seattle, WA; 2) Physiology and Biophysics, University of Washington, Seattle, WA; 3) Biology, Stanford University, Stanford, CA.
Kinetochores must form persistent, yet correctable attachments to dynamic microtubule tips, withstand tensile load, and correctly position chromosomes along the mitotic spindle. The highly conserved Ndc80 complex (Ndc80c) is a key microtubule-binding component of the kinetochore that is required for viability, but only weakly binds microtubules. Previous studies demonstrated that another microtubule-binding complex called the Dam1 complex (Dam1c), functions as a processivity factor for Ndc80c, enhancing its microtubule affinity, ability to track with dynamic tips, and increasing its capacity to remain attached under load. However, Ndc80c and Dam1c together cannot recapitulate the strength of native kinetochore particles purified from yeast. To determine if other kinetochore complexes contribute to kinetochore-microtubule attachment, we have reconstituted a stable interaction between Ndc80c and the central kinetochore complex, MIND, and demonstrate that they form a complex with a 1:1 stoichiometry. Using total internal fluorescence (TIRF) and optical trap microscopy, we found that MINDc directly enhances the binding strength of Ndc80c on microtubules. Intriguingly, the MIND complex binds Ndc80c far from its microtubule-binding domain, suggesting that MINDc allosterically enhances Ndc80c-microtubule interactions. We also demonstrate that the Dam1 and MIND complexes additively enhance the duration of Ndc80c microtubule binding and strengthen the microtubule attachment to nearly the level of native kinetochores.

33. Specialization of kinetochores for segregation of maternal and paternal chromosomes during meiosis I. ***Adele Marston**[1], Eris Duro[1], Krishna Sarangapani[2], Nadine Vincenten[1], Yi Deng[2], Kwaku Opoku[2], Flavia de Lima Alves[1], Juri Rappsilber[1], Qiaozhen Ye[3], Kevin Corbett[3], Sue Biggins[4], Charles Asbury[2]*. 1) Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, MIdlothian, United Kingdom; 2) Department of Physiology & Biophysics, University of Washington, Seattle, Washington 98195, USA; 3) Ludwig Institute for Cancer Research, San Diego CA, USA; 4) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109, USA.
The kinetochore plays a crucial role in chromosome segregation, serving as the microtubule attachment site and as an important signaling module that controls cell cycle progression via the spindle checkpoint. The chromosome segregation programme of meiosis I differs from the mitotic one because it is homologous chromosomes, rather than sister chromatids that are segregated. The kinetochore plays critical, but poorly understood, roles in setting up this modified segregation pattern. One unique feature of meiosis I is that homologous chromosomes are held together by chiasmata, the products of meiotic recombination, however, chiasmata formation is suppressed in centromeric regions. We show that kinetochore proteins suppress chiasmata formation within the pericentromere and that this suppression is critical to allow proper homolog segregation during meiosis I. Another important modification in meiosis I is that sister kinetochores are monooriented, that is they attach to microtubules emanating from the same spindle pole. In budding yeast, the meiosis I-specific protein complex monopolin has been proposed to achieve this by fusing sister kinetochores. To test this idea directly, we isolated native meiotic and mitotic kinetochore particles and reconstituted their function at the single particle level in vitro. Using laser trapping, we show that kinetochore particles from meiosis I form stronger attachments to dynamic microtubule tips than those from mitosis or meiosis II. Monopolin is both necessary and sufficient for the higher strength of kinetochores. Our data provide direct evidence that sister kinetochores are mechanically fused by monopolin during meiosis I so that microtubule-binding elements from the two sister kinetochores cooperate to form a single

attachment site. Overall, our findings uncover specialized roles of kinetochores that enable the segregation of maternal and paternal chromosomes during meiosis I.

34. An Asymmetric Competition for Protons Promotes Aging but Facilitates Rejuvenation. ***Kiersten A Henderson****, Adam L Hughes, Daniel E Gottschling.* Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.
Replicative aging in budding yeast is asymmetric - mother cells age but produce daughter cells with full lifespans. This suggests that the initiating events of aging and their resulting phenotypes will occur in mother cells but not be present in daughter cells. We recently discovered that acidity of the lysosome-like vacuole is asymmetric: vacuole acidity decreases in mother cells in early age and limits lifespan, but daughter cells regenerate vacuole acidity. What decreases vacuole acidity and how it is regenerated in daughter cells is unknown. Here we find that vacuole pH asymmetry is achieved through mother-daughter asymmetry of the major regulator of cytosolic pH, the plasma membrane proton ATPase (Pma1). We show that Pma1 is more abundant at the plasma membrane in mother cells than daughter cells. In addition, Pma1 accumulates in early age and its activity antagonizes vacuole acidity and limits lifespan. Conversely, low levels of Pma1 in daughter cells permit regeneration of vacuole acidity. Consistent with the distribution of Pma1, cytosolic pH increases during aging and is asymmetric between mother and daughter cells. These results support a model where high levels of Pma1 in mother cells reduce vacuole acidity by competing with the vacuolar proton ATPase for limited cytosolic protons. Moreover, the inherent mother-daughter asymmetry of Pma1 mediates regeneration of vacuole acidity in daughter cells by locally permitting higher cytosolic proton availability. Overall, our results identify asymmetric proton homeostasis as an early initiating event in aging that also underlies cellular rejuvenation.

35. The heterogeneity of peroxisomes in yeast. ***Sanjeev Kumar***[1,2]*, Ida J van der Klei*[1,2]. 1) Molecular Cell Biology, GBB, University of Groningen, Groningen, the Netherlands; 2) System Biology Center for Energy Metabolism and Ageing, Groningen, the Netherlands.
Peroxisomes are single membrane-bound organelles present in almost all eukaryotic cells. During mitosis in yeast, peroxisomes proliferate and are carefully partitioned over mother and daughter cell. In yeast, the major pathway of peroxisome proliferation is multiplication by fission of pre-existing organelles. We investigated whether we can discriminate between the older and younger peroxisomes *in vivo* using live cell fluorescence microscopy techniques and fluorescent proteins with different maturation times. This approach allows us to determine the age of the peroxisomal matrix protein content and thus of the peroxisomes. We used a fusion protein consisting of the slow maturing red fluorescent protein, DsRed1 (maturation half-life 11 h), and the fast maturing green fluorescent protein, superfolder GFP (sfGFP; maturation time ~10 min), containing a peroxisomal targeting signal. Analysis of yeast cells producing this fusion protein, revealed both young peroxisomes, which show only green fluorescence and old ones, which are both green and red fluorescent within one cell. Furthermore, we studied that how old and young peroxisomes differ in their capacity to import matrix and membrane proteins. By using a doxycycline-inducible system, we observed that older peroxisomes have a lower capacity of matrix protein import as compared to the younger ones. However, peroxisomal membrane proteins (PMPs) exhibited differences in their sorting to peroxisomes. Pex3, a PMP involved in membrane biogenesis, is sorted to all peroxisomes irrespective of their age, whereas, Pex14, a component of the receptor docking complex, is sorted only to the young ones. Further analysis revealed that as peroxisome become older the protein level of Pex14 decreased as compared to the younger ones. Live cell imaging confirmed that yeast cells contain a heterogeneous population of peroxisomes with respect to their age. Moreover, we observed that during budding the older peroxisomes are preferentially retained in the mother cell, while the daughter cell inherits relatively young ones. Furthermore, replicative lifespan analysis of peroxisome inheritance mutants using a microfluidics device revealed that *inp1* cells, which are unable to retain peroxisomes in the mother cell, are short lived, whereas, *inp2* cells, which retain all peroxisomes in the mother cell, have a longer replicative lifespan relative to wild type cells.

36. Translocation of cyclin C to the mitochondria mediates stress-induced fission and programmed cell death. ***Randy S. Strich****, Katrina F. Cooper.* Dept Molec Biol, Rowan University-SOM, Stratford, NJ.
The decision to undergo programmed cell death (PDC) is controlled by a complex interaction between nuclear and mitochondrial signals. In response to many stressors, yeast initiate a PCD pathway that exhibits many apoptotic hallmarks found in higher cells including loss of mitochondrial integrity and caspase activation. Mitochondria are highly dynamic organelles that constantly undergo fission and fusion. Studies have shown that one of the earliest PCD events is a dramatic shift in mitochondrial morphology toward fission. We have identified the transcription factor cyclin C as the biochemical trigger for stress-induced hyper-fragmentation. In response to PCD stimuli such as oxidative stress, cyclin C is released from the nucleus then associates with the fission machinery. Loss of cyclin C function prevents mitochondrial fission while ectopic introduction of cyclin C in the cytoplasm induces fission in the absence of stress. Recent studies have found that mitochondrial fission occurs at junctions between the mitochondria and the endoplasmic reticulum (ER). We have found that the number of these junctions increases upon stress at sites of mitochondrial fission. In addition, the formation of the additional stress-induced junctions requires cyclin C. The results suggest that cyclin C plays a role in establishing

and/or maintaining these stress-enhanced mitochondrial-ER junctions. Finally, although we have found a direct correlation between with the ability of the cell to undergo extensive mitochondrial fission and induce PCD, a mutation has been recovered in cyclin C that allows us to separate these two functions. These results suggest that cyclin C plays two independent roles in mediating fission and cell death. The first activity enhances association of the fission factor Dnm1p to the adaptor protein Mdv1p. Second, association of cyclin C to the mitochondria stimulates mitochondrial outer membrane permeability resulting in release of pro-apoptotic proteins sequestered in this organelle.

37. Engineering of alcohol and stress tolerance traits in *Saccharomyces cerevisiae* using a novel synthetic biology approach for producing genetic diversity. *Sabrina German[1], Biranchi N. Patra[2], Animesh Ray[2], **Helge Zieler**[1]*. 1) Primordial Genetics Inc., 3210 Merryfield Row, San Diego CA 92121; 2) School of Applied Life Sciences, Keck Graduate Institute, 535 Watson Drive, Claremont CA 91711.
Engineering of microbes for the production of fuels or chemicals from renewable feedstocks involves both rational genetic approaches, rooted in the understanding of gene function and genetic networks, and empirical approaches based on random genetic changes that produce desirable phenotypes through unanticipated mechanisms. Existing methods of both types are generally slow and often ineffective; they are also limited by the range of extant genetic diversity. To enable flexible optimization of microbes, new genetic technologies are needed that are relatively rapid, cost-effective, and able to produce desirable phenotypes including complex traits related to growth, stress resistance, and product tolerance.
We have developed Function Generator, a new genetic paradigm that creates novel biological functions by combining segments from different genes in a high throughput manner. The technology is designed to increase the phenotypic diversity of an organism and to achieve transferable phenotypes that are otherwise difficult to engineer. Large combinatorial libraries of synthetic genes that do not exist in nature are constructed from the protein coding content of an entire genome, or even multiple genomes. The technology is equally suited for well-studied organisms and for those characterized only by a genome sequence.
We applied this technology to develop stress and chemical tolerances in *Saccharomyces cerevisiae* by introducing a synthetic combinatorial library into a laboratory yeast strain and screening for survival and growth in the presence of ethanol, butanol, heat, salt and low pH. A variety of novel hybrid genes were isolated that confer resistance to one or more of these stresses. The novel synthetic coding sequences that impart tolerance to the selective conditions contain sequences from a variety of yeast genes, some with known involvement in stress tolerance and others with apparently unrelated or previously unknown functions.
The results of these studies show that Function Generator is suitable for enhancing product and stress tolerance in microbes, and suggest that this powerful new technology will be useful for engineering a variety of traits in any sufficiently transformable organism.

38. Mapping genetic suppression interactions on a global scale. ***Jolanda van Leeuwen**[1], Joseph Mellor[1,4], Guihong Tan[1], Takafumi Yamaguchi[1], Anastasia Baryshnikova[2], Paul Bansal[1,3], Michael Costanzo[1], Brenda Andrews[1,5], Frederick Roth[1,3,5], Charles Boone[1,5]*. 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, USA; 3) Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, Canada; 4) seqWell Inc, Beverly, MA, USA; 5) Department of Molecular Genetics, University of Toronto, Toronto, Canada.
Genetic suppression analysis is a powerful approach for identifying new and unexpected functional relationships between genes. Moreover, in the case of human disease genes, suppressors often represent strong candidates for therapeutic intervention. While genetic suppressors provide rich information about biological pathways, we do not yet understand the general principles that underlie genetic suppression networks. Here, we describe a large-scale study to identify spontaneous extragenic mutations that suppress fitness defects associated with deletion or conditional alleles in the model eukaryote, *Saccharomyces cerevisiae*. We used Synthetic Genetic Array (SGA) analysis to map the location of suppressor mutations to within ~200 kb. The identity of the suppressors is further characterized by next-generation sequencing. Thus far, we have identified and confirmed ~200 extragenic suppressor mutations, which encompass the full spectrum of adaptive mutations, including change- and gain-of-function mutations. The identified suppression interactions involve gene pairs that span a wide range of functional categories and include mutations in genes encoding mitochondrial ATPase subunits that suppress the growth defect associated with loss of mitochondrial translation. Most of the suppressor interactions we identified occur between functionally related genes and do not overlap with previously identified genetic or physical interactions. We are combining our new dataset with literature-curated suppression interaction data to construct a large-scale suppressor network and to compare the properties of the literature-curated subnetwork to the unbiased network mapped in this study. We plan to use the combined experimental and literature-curated networks to explore general rules that may apply to genetic suppression.

39. Toward a complete reference eukaryotic genetic interaction network. ***Benjamin VanderSluis**[1], Michael Costanzo[2,3], Elizabeth Koch[1], Carles Pons[1], Anastasia Baryshnikova[4], Wen Wang[1], Matej Usaj[2,3], Brenda J. Andrews[2,3], Charles*

Boone[2,3], *Chad L. Myers*[4]. 1) Department of Computer Science and Engineering, University of Minnesota-Twin Cities, Minneapolis, MN; 2) The Department of Molecular Genetics, University of Toronto, Toronto ON; 3) Donnelly Centre for Cellular and Biomolecular Research Toronto, ON; 4) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.
Genetic interactions provide rich information about gene function and broad cellular organization. We provide an update on our on-going effort to map the complete genetic interaction network for *Saccharomyces cerevisiae* using Synthetic Genetic Array (SGA) analysis and a colony size-based readout. To date, we have screened mutants in more than 4828 unique genes against arrayed collections of deletion mutants for nonessential genes (~4000) and/or temperature-sensitive (TS) alleles of essential genes (~700). This network represents a ~4-fold increase in the number of gene pairs investigated relative to our previous study, and the resulting genetic interaction network consists of more than 700,000 quantitative negative and positive genetic interactions connecting more than 85% of all yeast genes. Importantly, because our network includes interactions for more than 1000 temperature-sensitive alleles covering more than 700 essential genes, we can assess genetic interactions between pairs of essential genes as well as interactions connecting essential and non-essential genes. As we approach completion of the first draft of a reference network, we can now characterize the broad principles that govern genetic interactions and how these principles relate to other molecular networks or genes functions. We see striking contrasts between the non-essential and essential genetic interaction networks, both in terms of their topology in relation to other molecular networks, and in the connectivity of particular cellular functions. As expected, the essential genetic interaction network is denser than the non-essential network. Importantly, the structure and functional characteristics of individual interactions suggest key differences in the type of information genetic interactions relay. More generally, we demonstrate that the complete yeast genetic interaction network has direct relevance to our understanding of disease heritability. Specifically, structural and topological features associated with the yeast genetic network can be leveraged to identify genetic interactions underlying complex diseases in humans.

40. Global analysis of HAT and HDAC substrates in vivo. ***David Paul Toczyski***, *Michael Downey.* Biochemistry, UCSF, San francisco, Ca.
While histone acetylation and deacetylation machineries (HATs and HDACS) regulate important aspects of cell function by targeting histone tails, recent work highlights that non-histone protein acetylation is also pervasive in eukaryotic species. HATs and HDACs that also regulate non-histone protein acetylation have roles in preventing a plethora of human syndromes and disease states, with sirtuin HDACS receiving considerable attention as regulators of metabolic and genomic stability. Yet, how sirtuins cooperate with each other and with multi-subunit acetyltransferases to regulate cell physiology on a global scale is unclear. Here we use a quantitative mass-spectrometry approach to define the acetylation landscape of a yeast strain mutated for multiple sirtuin enzymes previously impacted in the regulation of non-histone protein acetylations. We also use our approach to define the roles of HATs in regulating sirtuin substrates and to investigate the role of Ada2 protein in regulating Gcn5 HAT function. Finally, we show that the Esa1 HAT and sirtuin-regulated sites on the ribosomal protein gene transcription factor Sfp1 are important for the cellular response to TOR inhibition by the drug rapamycin.

41. Protein localization re-patterning during DNA replication stress. ***Nikko P. Torres***, *Grant W. Brown.* Department of Biochemistry and Donnelly Centre, University of Toronto, 160 College St., Toronto, Ontario, Canada M5S 3E1.
Subcellular reorganization of proteins is a hallmark of the cellular response to DNA replication stress. We use high-throughput microscopy to develop a systems-level view of the re-patterning of fluorescently-tagged proteins during chemically-induced DNA replication stress. A combination of manual and automated analyses of over 4000 GFP-tagged proteins reveals drug-specific protein relocalization signatures. By imaging the 267 proteins that change in their subcellular location over time, we recently determined that many proteins exhibit drug-specific re-patterning kinetics. We are now probing the regulation of localization re-patterning during replication stress by disrupting DNA damage response (DDR) signaling. We deleted the DDR kinase Rad53 and found that a third of protein re-localizations were dependent on Rad53, suggesting a central role for checkpoint kinases in regulating protein re-patterning in response to DNA replication stress. In addition to regulating nuclear localizations, we found that Rad53 important for drug-specific P-body formation in the cytoplasm. Together, our data suggest that checkpoint kinase-dependent phosphorylation regulates diverse protein localizations.

42. High dimensional phenotyping reveals remarkable extent of haploinsufficiency in essential genes. ***Shinsuke Ohnuki***, *Yoshikazu Ohya.* Department of Integrated Biosciences, University of Tokyo, Kashiwa, Chiba, Japan.
Heterozygous is defined as having two different alleles for the same trait. A loss-of-function mutation in heterozygous diploid is thought to confer a phenotype rarely, because another intact gene allele complements the loss-of-function allele in most case. This phenomenon is called haploinsufficiency which is known to have relevance to a human disease. In *Saccharomyces cerevisiae*, ~3% of 5,900 genes were identified as haploinsufficient for growth in rich media, where an insufficient protein production was suggested to the primary mechanisms of the haploinsufficiency.

In this study, high dimensional phenotyping was performed for 1,112 essential heterozygous diploids. We quantified 501 morphometric traits in each heterozygous mutants based on fluorescent microscopic images with image processing program CalMorph, and defined relevant probability distribution models for each trait to detect morphometric abnormality against that of wild-type. We found that 584 (52%) out of 1,112 heterozygous diploids showed abnormal phenotypes at FDR = 0.005 (P 3.1×10^{-5}). To compare the extent of haploinsufficiency of non-essential genes, we tested randomly-selected 100 non-essential gene deletion mutants. Twenty-six heterozygous diploids (26%) showed haploinsufficiency in at least one trait, suggested that a large extent of haploinsufficiency observed in the heterozygous essential gene deletion mutants is due to their essential roles for viability.
To clarify the gene functions related to the haploinsufficient morphometric phenotypes, we analyzed the correlation between morphometric profiles and gene functions. By canonical-correlation analysis, we found that 607 (55%) out of 1,112 genes mediated the correlation between the morphometric phenotypes and the gene functions. This implies that more than half of the genes involved in various biological processes were related to the haploinsufficient phenotypes. For example, decreased gene dosage of chaperonin CCT complex and RNA polymerase conferred variability on nuclear shapes and cell elongation, respectively. We also gave a global view of the gene network based of the phenotypic similarity. Genes responsible for the distinct biological process were clustered in the network, indicating that morphometric similarity of the heterozygous diploid can be used to visualize a phenotypic landscape of the essential genes.

43. Intrinsically disordered proteins drive heritable epigenetic switches that transform the phenotypic landscape of *S. cerevisiae*. *Sohini Chakrabortee[2], James Byers[1], Susan Lindquist[2],* ***Daniel Jarosz***[1]. 1) Stanford University, Stanford, CA; 2) Whitehead Institute, Cambridge, MA.
Transient over-expression of individual proteins can drive the appearance of heritable new biological traits, as exemplified by yeast prion proteins. To examine the breadth of this phenomenon we over-expressed virtually all *Saccharomyces cerevisiae* open reading frames individually and transiently. Strikingly, bursts in the expression of over fifty intrinsically disordered proteins (IDPs) created heritable new traits that persisted for hundreds of generations after over-expression was stopped. The inheritance of these traits resembled known prions in several ways. Transmission from one generation to the next depended on protein homeostasis networks and was non-Mendelian in character. However, unlike most known yeast prions, their inheritance did not depend upon the Hsp104 disaggregase, but instead required other molecular chaperones (Hsp70 or Hsp90). Moreover, these IDPs generally lacked Q/N-rich sequences and did not form high molecular weight amyloid fibers. Several of the heritable epigenetic states conferred gain-of-function phenotypes, and most improved growth in a wide variety of environmental stresses. We also observed a high incidence of such protein-based inheritance in wild yeast strains. Thus, these newly identified prions and stable phenotypes provide novel mechanisms of protein-based inheritance that can fuel the acquisition of adaptive traits in nature.

44. Cheaters Do Prosper: Reciprocal cheating drives epigenetic switching of facultative multicellularity. *Sorna Kamara,* ***Randal Halfmann***. Dept Biochemistry, UT Southwestern Medical Ctr, Dallas, TX.
Cooperation between genetically identical cells is the basis of multicellularity. Despite an extensive theoretical framework, the molecular mechanisms that engender the evolution of cooperation remain enigmatic. We demonstrate that prions and other switch-like mechanisms that regulate yeast cell surfaces suffice to create stable divisions of labor within genetically homogeneous populations. These switches produce phenotypic granularity, with discrete subpopulations differing according to whether or not they express the adhesin, Flo11. Cells that express Flo11 exhibit a wasteful metabolic strategy in which tremendous resources are expended on the production of a secreted lubricant that enables them to slide into uncolonized territory. Doing so allows them to secure more than their fair share of common resources, albeit at the expense of the more-efficient, non-Flo11 expressing cells and therefore to the detriment of the population as a whole. Counter to theoretical expectations, we observe that non-Flo11-expressing cells nevertheless persevere, in part because they too are mobilized by the surfactant produced by Flo11-expressing cells. Moreover, they exhibit a conservative metabolic strategy that takes advantage of the nutritional niche created in the wake of Flo11-driven colonization. Thus, cheating by one cell type entails the production of a new public good, which is in turn exploited by the second cell type. Epigenetic switching between the two cell types enables an individual genome - and each of the individual genes that promotes switching - to benefit from both the original and reciprocal forms of cheating. The net result is a differentiated population that resists exploitation by unrelated individuals.

45. The PUF Protein Puf3 Toggles the Translational Fate of Bound mRNAs to Regulate Mitochondrial Biogenesis. ***Chien-Der Lee****, Benjamin Tu*. Biochemistry Dept, UTSW, Dallas, TX.
PUF proteins are conserved post-transcriptional regulators that bind in a sequence-specific manner to the 3UTRs of mRNAs. Paradoxically, PUF proteins have been proposed to promote both degradation of their target mRNAs and their translation. Herein, we show how a yeast PUF protein Puf3p responds to glucose availability to switch the fate of its target mRNAs that encode proteins required for mitochondrial biogenesis. Upon glucose depletion, Puf3p becomes

phosphorylated, associates with polysomes, and actively promotes translation of its target mRNAs. Strikingly, a Puf3p mutant that prevents its phosphorylation cannot promote mRNA translation and becomes trapped in intracellular PUF-bodies that dominantly poison the cell in a mRNA-dependent manner. Our results reveal that nutrient-responsive phosphorylation toggles the activity of Puf3p to promote either degradation or translation of pre-existing mitochondrial mRNAs. Such activation of mRNA translation might enable rapid adjustment to environmental changes without the need for de novo transcription.

46. Genome-wide screen identifies pathways that govern tRNA splicing and intron turnover in Saccharomyces cerevisiae. ***Jingyan Wu***, *Yao Wan, Anita Hopper*. Department of Molecular Genetics, The Ohio State University, Columbus, OH.
tRNAs are essential for protein synthesis in all kingdoms of life. In yeast, tRNA biogenesis involves removal of the 5 and 3 ends, addition of CCA and modifications, and for tRNAs encoded by intron-containing genes, splicing of introns. In yeast and mammalian cells, the subcellular movement of tRNAs is bidirectional between the nucleus and the cytoplasm. However, many aspects of tRNA metabolism and subcellular movement remain unknown. For example, the mechanisms that regulate tRNA splicing and intron turnover are poorly understood. To identify all the missing players involved in tRNA biology, we conducted a systematic and unbiased screen of the whole yeast genome using the yeast deletion and temperature-senstive (ts) collections, that together provide mutant alleles for 90 percent of all annotated genes. We developed a new Northern method that allows rapid and sensitive analysis of mature tRNAs and their processing intermediates. The complete set of 4848 deletion strains and 765 strains with ts mutations of essential genes were analyzed for defects in tRNA biology. Genetic and biochemical analyses of some of the identified mutants have provided surprising insights. For instance, deletion of TOM70 and SAM37 cause tRNA splicing defects and accumulation of end-matured intron-containing tRNAs. We learned that the tRNA splicing defects occur because Tom70 and Sam37 are required for proper localization, assembly, and function of the subunits of the tRNA splicing endonuclease complex onto the mitochondrial surface. We also learned that deletion of XRN1, encoding the cytoplasmic 5 to 3 exonuclease, causes accumulation of free tRNA introns. Further investigation uncovered, for the first time, the mechanism of tRNA intron turnover; surprisingly, it is a multi-step process requiring cooperation between tRNA ligase, Rlg1, and Xrn1. Numerous additional mutations that affect other aspects of tRNA biology were identified. Thus, our genome-wide screen has led to discoveries of novel gene products that function in eukaryotic tRNA transcription, processing, and subcellular dynamics.

47. Heritable variation of mRNA decay rates in yeast. ***Jennifer M Andrie***, *Jon Wakefield, Joshua M Akey*. Genome Sciences, University of Washington, Seattle, WA.
Gene expression levels are determined by the balance between rates of mRNA transcription and decay, and genetic variation in either of these processes can result in heritable differences in transcript abundance. Although the genetics of gene expression has been the subject of intense interest, the contribution of heritable variation in mRNA decay rates to gene expression variation has received far less attention. To this end, we developed a novel statistical framework and measured allele-specific differences in mRNA decay rates in a diploid yeast hybrid created by mating two genetically diverse parental strains. In total, we estimate that 52% of genes exhibit allelic differences in mRNA decay rate, of which 518 can be identified at a false discovery rate of 5%. Genes with significant allele-specific differences in mRNA decay rate have higher levels of polymorphism compared to other genes, with the 5 UTR region particularly diverse. Strikingly, we find widespread evidence for compensatory evolution, such that variants influencing transcriptional initiation and decay having opposite effects, suggesting steady-state gene expression levels are subject to pervasive stabilizing selection. Our results demonstrate that heritable differences in mRNA decay rates are widespread, and are an important target for natural selection to maintain or fine-tune steady-state gene expression levels.

48. A Stable Quasi-Filamentous Growth Pattern in Budding Yeast. *Junwon Kim,* ***Mark D. Rose***. Molecular Biology, Princeton University, Princeton, NJ.
Upon nutrient limitation, budding yeast can adopt alternate filament-like growth patterns called diploid pseudohyphal or haploid invasive growth. We report a novel robust quasi-filamentous growth state, sharing some characteristics with classic forms of filamentous growth, but differing in crucial aspects of morphology, growth conditions and genetic regulation. Quasi-filamentous growth comprises flocculent chains of highly elongated cells in liquid rich media, requiring neither starvation, nor Flo8p and Flo11p, and occurs in haploid S228C strains. Quasi-filamentous growth arises in *fus3* mutants together with various mutants affecting normal septin assembly. The cells undergo prolonged G2 delays, while polarized bud growth continues, dependent on Swe1p. The highly elongated quasi-filamentous cells contain an apical Spitzenkörper, characteristic of filamentous fungi. Quasi-filamentous growth is induced by defects in mitotic septin assembly, requires signaling through Ras2p and the MAPK Kss1p, and is repressed by Fus3p. Unlike pseudo-hyphal growth, quasi-filamentous growth requires the scaffold protein Ste5 and the G-protein / from the pheromone response pathway. The *swe1* mutation abolished both Kss1p-signaling and filamentous growth suggesting that a positive feedback loop amplifies and maintains quasi-filamentous growth. Taken together, our findings show that budding yeast can stably access a radically altered growth pattern with very few genetic changes.

49. Gradient tracking in yeast: role of G protein polarity. *Allison McClure[1], Jayme Dyer[1], Maria Minakova[2], Timothy Elston[2], Daniel Lew[1]*. 1) Pharmacology and Cancer Biology, Duke University, Durham, NC; 2) Pharmacology, University of North Carolina, Chapel Hill, NC.
Many cells are extremely proficient at tracking chemical gradients. While motile cells like neutrophils exhibit directed cell migration in response to a chemical gradient (chemotaxis), yeast are nonmotile, and instead direct polar growth up a chemical gradient (chemotropism). During mating, two haploid yeast cells of different mating types grow towards one another until they fuse and become a diploid. The cells respond to their mating partners pheromone via a G protein-coupled pheromone receptor (GPCR) pathway that biases the location of the master regulatory Rho-GTPase, Cdc42. Cdc42 orients actin cables, and therefore growth, up-gradient. During gradient tracking, Cdc42 and other associated polarity proteins form a patch that wanders around the cell cortex (1). Pheromone signals act to constrain the wandering, so the polarity patch spends more time on the up-gradient side of the cell, enabling gradient tracking. Here we address the mechanism whereby GPCRs constrain polarity patch wandering. To constrain patch wandering, pheromone-activated G must interact with the Cdc42-directed GEF: mutants that disrupt this interaction display dramatic wandering and loss of chemotropism (1,2). We show using computational modeling that in order to constrain wandering, the G-associated GEF must itself be polarized. This provides a rationale to explain the previous observation that pheromone receptors are polarized (2). By polarizing their receptors, cells would also polarize G and the associated GEF in order to constrain wandering. However, we found that mutant receptors that cannot become polarized were still proficient at constraining wandering. Surprisingly, G was still polarized in cells with the mutant receptor, demonstrating that G can polarize even in the absence of receptor polarity. Further, we found that some G co-localizes with endosome markers and has a slow FRAP recovery, suggesting G recycles by a vesicle trafficking mechanism. We suggest that cells tracking a gradient polarize their G protein signaling in order to constrain polarity patch wandering on the up-gradient side of the cell. 1.Dyer, J. et al. (2013) Curr Biol 2.Arkowitz, RA. (2009) Cold Spring Harb Perspect Biol.

50. Spatial control of microtubule length and lifetime by opposing stabilizing and destabilizing functions of Kinesin-8. *Yusuke Fukuda, Anna Luchniak, Erin Murphy, **Mohan Gupta***. University of Chicago, Chicago, IL.
Microtubules (MTs) are dynamic cytoskeletal filaments that are essential for intracellular organization and cell division. To function in diverse and complex cellular processes, the dynamic behavior of MTs must be differentially regulated within the cell. Yet, the mechanisms that spatially and temporally control MT dynamics are largely unclear. In budding yeast, the spindle position checkpoint (SPOC) inhibits mitotic exit in response to mispositioned spindles. To maintain SPOC-mediated anaphase arrest, astral MTs must maintain persistent interaction with the bud neck. However, the molecular mechanisms that maintain this interaction are not known. We demonstrate that persistent interaction of astral MTs with the bud neck is achieved by spatial control of MT catastrophe and rescue, which extended MT lifetime 25-fold and controlled the length of dynamic MTs within the bud compartment. Moreover, the single kinesin-8 motor protein, Kip3, alternately mediated both catastrophe and rescue of the bud MT. Kip3 accumulated in a length-dependent manner along MTs specifically within the bud compartment. Yet, on these MTs Kip3 induced catastrophe spatially at the bud tip, independent of MT length. Rather, this accumulation of Kip3 facilitated its association with the ends of depolymerizing MTs, where Kip3 promoted rescue before MTs exited the bud. MT rescue within the bud required the tail domain of Kip3, whereas the motor domain mediated catastrophe at the bud tip cortex. In vitro, Kip3 exerted both stabilizing and destabilizing effects on reconstituted yeast MTs. Together these data demonstrate that the kinesin-8 Kip3 is a multifunctional regulator that alternately stabilizes and destabilizes specific MTs. Control over MT catastrophe and rescue by Kip3 defined the length and lifetime of MTs within the bud compartment. Furthermore, we found that this subcellular regulation of MT dynamics by Kip3 is critical to maintain mitotic arrest in response to mispositioned spindles.

51. Polarization of the Endoplasmic Reticulum by ER-Septin Tethering. ***Jesse T. Chao[1]**, Andrew K.O. Wong[1], Shabnam Tavassoli[1], Barry P. Young[1], Adam Chruscicki[2], Nancy N. Fang[2,3], LeAnn J. Howe[2], Thibault Mayor[2,3], Leonard J. Foster[2,3], Christopher J.R. Loewen[1]*. 1) Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada; 2) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada; 3) Centre for High-Throughput Biology, University of British Columbia, Vancouver, BC, Canada.
Polarization of the plasma membrane (PM) into domains is an important mechanism to compartmentalize cellular activities and to establish cell polarity. Polarization requires formation of molecular diffusion barriers that prevent mixing of proteins between domains. Recent studies have uncovered that the endoplasmic reticulum (ER) of budding yeast and neurons is polarized by diffusion barriers, which in neurons controls glutamate signaling in dendritic spines. The molecular identity of these diffusion barriers is currently unknown. Here we show that a direct interaction between the ER protein Scs2 and the septin Shs1 creates the ER diffusion barrier in yeast. Barrier formation also requires Epo1, a novel ER-associated subunit of the polarisome that interacts with both Scs2 and Shs1. ER-septin tethering polarizes the ER into separate mother and bud domains, one function of which is to regulate the timing of the molecular events in nuclear positioning. The ER diffusion barrier restricts the spindle capture protein Num1 to the mother ER, resulting in the spindle

to be kept in the mother until nuclear division. Therefore, this work encompasses the molecular dissection of the ER diffusion barrier and the demonstration of an important physiological role of the barrier.

52. Coordination between terminal septin subunits: both Cdc11 and Shs1 promote bud neck recruitment of the myosin II-binding factor Bni5. ***Gregory C. Finnigan****, Julie Tagaki, Christina Cho, Elizabeth Booth, Jeremy Thorner.* Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA.
Septins are a family of GTP-binding proteins that are considered cytoskeletal elements because they assemble into a rod-shaped hetero-octameric building block that is able to polymerize into higher-order filamentous structures, and because the resulting structures form cellular barriers and scaffolds. In mitotic *S. cerevisiae* cells, five septin subunits associate to form either Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11 or Shs1-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Shs1 hetero-octamers. These linear apolar complexes can self-organize, both in vivo and in vitro, into diverse supramolecular ensembles, including long paired filaments, arcs, rings, spirals, gauze-like arrays, bundles, sheets, and hourglass-shaped forms. Septins have been implicated in a number of biological processes in yeast, including bud site selection, recruitment of bud neck-associated factors, cell cycle regulation, spindle positioning, cell morphogenesis, membrane remodeling, and cytokinesis. However, the molecular mechanisms by which septin-based structures recruit other factors has been unclear. We have been investigating the molecular interplay of the two most recently evolved septin subunits, Cdc11 and Shs1, and how they contribute to septin architecture and function during vegetative growth. Comprehensive genetic analysis has revealed multiple conditions under which Shs1 becomes essential for viability and have allowed us to establish that Shs1 has a function(s) that partially overlaps with Cdc11, in keeping with the fact that each of these subunits occupies the terminal position within the hetero-octamer. Moreover, we demonstrate that the C-terminal extension (CTE) element in Shs1 and Cdc11 is necessary and sufficient for the function of Cdc11 and Shs1, and plays a distinct cellular role from the CTEs in the other CTE-containing subunits (Cdc3 and Cdc12). Finally, we were able to show that constraining Bni5 by other means to the bud neck suppresses CTE mutants of Cdc11 and Shs1, and that a *bni5* mutation enhances the phenotypes of *cdc11* and *shs1* loss-of-function alleles. Furthermore, optimal Bni5-GFP localization to the bud neck requires the CTE of Cdc11 or Shs1. These findings indicate at least one function shared by the CTEs of Cdc11 and Shs1 is recruitment to the bud-neck of Bni5, which is a factor necessary for tethering the Myo1 (myosin II) required for formation of the actomyosin contractile ring that executes plasma membrane constriction during cytokinesis.

53. A cytosolic chaperone network mediates quality control of higher-order septin assembly. *C. Johnson[1], A. Weems[1], J. Brewer[2], J. Thorner[2],* ***M. McMurray***[1]. 1) Cell and Developmental Biology, Univ Colorado Anschutz Medical Campus, Aurora, CO; 2) Molecular and Cell Biology, Univ California, Berkeley, CA.
Septins are GTP-binding proteins that form hetero-oligomers and assemble into cytoskeletal filaments with essential functions in *S. cerevisiae*. Mutations located within the subunit-subunit interaction surface that encompasses the GTP-binding pocket in each septin (its "G interface") cause, in yeast, thermo-instability in septin hetero-oligomer assembly and, in humans, disease. We found that, when such a G interface mutant allele is co-expressed with the wild-type allele of the same septin, only the wild-type subunit is incorporated into filamentous structures, even at moderate temperatures, indicating that a mechanism for quality control exists. We show here that preferential selection of the wild-type subunit occurs during *de novo* synthesis and assembly of the hetero-octameric rod that is the building block of septin filaments. We demonstrate further that deletion of any of several specific chaperone-encoding genes abrogates the observed bias for the wild-type subunit and, conversely, that over-expression of the same chaperones lowers the temperature at which cells remain viable when they express a G interface mutant as the sole source of a given septin. These genetic findings strongly suggest that mutations that destabilize the G interface (but not septin mutations elsewhere in a subunit that cause with equally severe temperature-sensitive phenotypes) retard release of the mutant septin from these cytosolic chaperones, whereas the wild-type protein is not sequestered. Our results indicate, first, that the striking quality control we observed in septin assembly is exerted because, unlike the wild-type subunit, a G interface mutant experiences a protracted kinetic delay in escape from the chaperones that assist its proper folding during biogenesis and, second, that guanine nucleotide serves as an important cofactor for efficient septin folding. These findings may help explain the underlying etiology of those human diseases that arise from G interface mutations in human septins.

54. Protein based interference of Ty1 retrotransposition in budding yeast: characterization of copy number control resistant mutants. ***J.A. Mitchell****, A. Saha, Y. Nishida, M. Larango, L. Wachsmuth, E. Talevich, N. Kannan, D.J. Garfinkel.* Biochemistry and Molecular Biology, University of Georgia, Athens, GA.
The *Saccharomyces* LTR retrotransposon Ty1 is a mobile genetic element whose life cycle resembles retroviral replication. Ty1 elements contain *GAG* and *POL* genes, which are translated to produce Gag, the structural protein of virus-like particles (VLPs), integrase (IN), reverse transcriptase (RT), and protease (PR). Interestingly, Ty1 transposition decreases as the copy number increases, a system referred to as copy number control (CNC). A key feature of CNC is the loss of mature IN and reduced levels of RT. Recently, our lab discovered that poly(A)$^+$ 5 truncated Ty1 transcripts are expressed in several standard lab strains. The 5 truncated transcript initiates within GAG and encodes an N-terminal

truncated Gag called p22, which confers a potent trans-dominant negative phenotype on Ty1 mobility. These and additional analyses show that p22 is necessary and sufficient for CNC. To examine the molecular target of CNC, we are characterizing Ty1 mutants that are resistant (CNC^R) to the inhibitory effects of p22. CNC^R Ty1 elements contain missense mutations in *GAG*, with most mutations clustering within conserved domains of Pseudoviridae Gag proteins. One CNC^R Gag mutation (I201T) rescues the level of mature IN in VLPs, which suggests that Gag mediates the inhibitory properties of p22. Several other lines of evidence suggest that p22 is incorporated into VLPs, including co-localization of Gag and p22 in retrosomes and the formation of another novel protein, p18, which likely results from processing of p22 by PR. Interestingly, CNC^R mutants exhibit reduced processing of p22, consistent with the idea that less p22 is successfully incorporated into VLPs. Perhaps p22 is incorporated into VLPs via association with Gag and disturbs normal VLP function. Following this logic, CNC^R mutations might disrupt Gag and p22 protein-protein interactions. Present efforts are aimed at confirming a physical interaction between p22 and Gag, while future studies will investigate the consequence of p22 incorporation on VLP formation and maturation.

55. The impact of polyploidy on the rate and dynamics of adaptation. ***Anna M. Selmecki***[1], *Yosef E. Maruvka*[2], *Philip A. Richmond*[3], *Marie Guillet*[1], *Noam Shoresh*[4], *Amber Sorenson*[3], *Subho De*[2], *Roy Kishony*[4], *Franziska Michor*[2], *Robin Dowell*[3], *David Pellman*[1]. 1) Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 2) Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; 3) BioFrontiers Institute, University of Colorado, Boulder, CO; 4) Systems Biology, Harvard Medical School, Boston, MA.
Polyploidy is observed across the tree of life, yet its influence on evolution remains cryptic. In particular, the mechanism of how polyploidization impacts the rate and dynamics of adaptation is unclear. We performed in vitro evolution experiments in isogenic haploid, diploid, and tetraploid S. cerevisiae populations to analyze the acquisition of beneficial mutations. Compared to haploid and diploid populations of the same size, tetraploid populations displayed a rapid spread of mutations throughout the population. Mathematical modeling predicted that this rapid tetraploid adaptation was driven by higher rates of beneficial mutations and increased fitness effects of the acquired mutations. These predictions were supported by whole-genome sequencing and phenotypic analyses of clones from the evolved populations. Chromosome aneuploidy, concerted chromosome loss, and point mutations provided large, tetraploid-specific fitness gains. Our data support the importance of the polyploid state for the rapid generation of genetically diverse progeny, thereby driving adaptation to adverse environments. Our data provides the first quantitative evidence to support a driving role for polyploidy and genomic instability in evolution.

56. Polyploidy drives population heterogeneity through random and stepwise chromosome loss. ***Meleah A. Hickman***, *Carsten Paulson, Judith Berman*. Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.
The opportunistic pathogen *Candida albicans*, is often described as an asexual yeast and obligate diploid, yet has a large repertoire of mechanisms to generate genetic and phenotypic diversity despite the lack of a true meiosis in its lifecycle. The parasexual cycle enables shifts in ploidy, which in turn, facilitates recombination, aneuploidy and homozygosis of whole chromosomes to fuel rapid adaptation. Here we show that the tetraploid state potentiates ploidy variation and drives population heterogeneity. Tetraploidy is intrinsically stressful to *C. albicans* cells and the rate of loss of a heterozygous marker (LOH) increases by over an order of magnitude relative to diploid cells. Isolates recovered after LOH selection were viable and had highly aneuploid DNA contents ranging between diploid and tetraploid. Sequence analysis of these isolates with unconventional ploidies revealed a broad range of karyotypes including strains with a combination of di-, tri and tetra-somic chromosomes. These results suggest that chromosome loss process of the parasexual cycle appears to be stochastic and that few, if any, chromosomes are lost coordinately. Using a combination of high-throughput technologies, sequencing, and long-term experimental evolution, we analyzed the ploidy trajectories of several hundred tetraploid- and aneuploid-derived isolates and their fitness consequence. Both the tetraploid and aneuploid isolates resolved their genomes to a stable euploid state, that for the majority of isolates was the canonical diploid state. However, stable triploid and tetraploid states were observed in ~30% of the isolates. Notably, the isolates starting aneuploid were highly unstable and ~50% of them settled into a stable euploid state after only four days of passaging. In contrast, very few tetraploid isolates had changed ploidy after four days of passaging, but nearly 90% of them had changed ploidy within 28 days. Together our results indicate that tetraploid progenitors can produce progeny cells with a high degree of genomic diversity within a single population, and, as such, are an excellent source of genetic variation upon which selection can act.

57. Complex genetic interaction profiles reveal evolutionary fates of duplicated genes. ***Elena Kuzmin***[1,2], *Benjamin VanderSluis*[4], *Yiqun Chen*[2], *Raamesh Deshpande*[4], *Matej Usaj*[2], *Alex Nguyen*[3], *Alan Moses*[3], *Michael Costanzo*[2], *Chad L. Myers*[4], *Brenda J. Andrews*[1,2], *Charles Boone*[1,2]. 1) Molecular Genetics, University of Toronto; 2) Donnelly Centre for Cellular & Biomolecular Research, Toronto, Canada; 3) Cell & Systems Biology. Ecology, Evolutionary Biology & Computer Science, University of Toronto; 4) Computer Science & Engineering, University of Minnesota-Twin Cities, Minneapolis, USA.
The Saccharomyces cerevisiae genome retained 551 genes after whole genome duplication and the deletion of 35% of

these sister paralogs results in a synthetic sick or lethal (SS/SL) genetic interaction, suggesting that they buffer each others loss. The remaining duplicates do not show an SS/SL and may be redundant with a third gene, which masks their functional relationship. The digenic interaction profiles of individual paralogs provide insight about their degree of functional divergence; whereas, the functions that are shared between the paralogs can be interrogated through their trigenic interaction profiles. We are using an automated form of yeast genetics called Synthetic Genetic Array (SGA) analysis to explore trigenic interactions involving paralog pairs for the purpose of understanding paralog gene function and testing models of how paralog pairs are maintained through evolution. In its simplest form, the fate of duplicated genes has been either one of functional divergence, for functional specialization by sub- or neo-functionalization, or functional redundancy, for back-up compensation or dosage amplification. To differentiate between these possibilities, we are generating genetic interaction profiles for single mutants of sister paralogs and double mutants lacking both paralogs. So far we have analyzed over 250 paralog pairs and we have identified a functionally divergent set, as well as a set displaying a gradient of trigenic interaction frequency, suggesting that these paralogs retained varying degrees of functional redundancy. Physiological and evolutionary features, such as the double mutant fitness defect, average digenic interaction degree, expression level, rate of divergence and coding sequence similarity, correlate with trigenic interaction frequency and can be used for its prediction using a machine learning algorithm. Thus, the assessment of trigenic interactions offers a novel approach to study the functional relationship and buffering capacity of sister duplicates.

58. Sexual conflicts and chromosome rearrangements drive infertility. ***Sarah E. Zanders***, *Michael Eickbush, Jonathan Yu, JiWon Kang, Gerry Smith, Harmit Malik.* Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.
Meiosis generates haploid gametes (e.g. sperm) with only one copy of each chromosome from diploid progenitor cells that contain two copies of each chromosome. Because homologous alleles are no longer contained within the same genome after meiosis, there is evolutionary incentive for homologous alleles to compete for preferential inclusion in viable gametes. This competition can lead to the evolution of meiotic drive alleles that cheat during meiosis or during gamete maturation to be transmitted into more than 50% of the viable gametes of a heterozygote. Meiotic drive can be both directly and indirectly costly to fitness, so natural selection will favor the evolution of suppressors of the selfish drive behavior. The genetic conflicts between selfish meiotic drive alleles and their suppressors have been proposed to set off a molecular arms race in which both sides rapidly evolve to try and gain an upper hand. This evolution could potentially drive changes in key pathways that act in gametogenesis and contribute to infertility within a species and to the genetic isolation of different species. We developed a novel system utilizing hybrids of the fission yeasts S. pombe (Sp) and S. kambucha (Sk) to characterize how natural variation can contribute to infertility. Sp and Sk are 99.5% identical at the DNA sequence level, but Sk/Sp hybrids are largely infertile. We find that chromosomal rearrangements and related recombination defects are major causes of hybrid infertility. These chromosomal rearrangements are each linked to meiotic drive alleles, one per chromosome in Sk. These selfish alleles independently contribute to hybrid infertility by causing nonrandom death of spores that do not inherit them. Our study provides support for models in which genetic conflicts can drive infertility and speciation.

59. 3D structure of yeast synthetic chromosomes. ***Heloise Muller***[1,2], *Axel Cournac*[1,2], *Romain Koszul*[1,2]. 1) Genomes and Genetics, Institut Pasteur, Paris, France; 2) CNRS UMR3525, F-75015 Paris, France.
Sc2.0 project aims at assembling a synthetic yeast genome containing edits compared to the yeast Saccharomyces cerevisiaes native genome. So far, several synthetic chromosomes have been assembled, including chromosome *synIX_R* and *synIII* that has been recently published (Annaluru N, Muller H *et al*., science 2014). Specifically, the Sc2.0 design include a rapid evolution system, called SCRaMbLE, allowed by the insertion of loxPsym sites all along the chromosomes. Turning this system ON by expressing the CRE recombinase allows the apparition of multiple structural variants in one experiment. On top of this evolution system, Sc2.0 design include other specificities, like deletion of transposons and subtelomeres, or relocation of tRNA genes which can also be involved in general chromosome organization. Using Chromosome Conformation Capture (3C) experiments, we can determine the 3D conformation of chromosomes and determine changes compared to the WT strain. Here we describe the 3D structure of some SCRaMbLE structural variants in order investigate the potential relationships between linear and 3D organization of genome. This structure is also being analyzed in regards to the replication profile of the strain, as some variants have deleted or relocated ARS sequences.

60. Towards in vivo NMR: NMR of prion fibrils at endogenous levels in cellular lysates. ***Kendra K Frederick***[1], *Vladimir K Michaelis*[2,3], *Björn Corzilius*[2,3], *Ta-chung Ong*[2,3], *Jacavone Angela*[2,3], *Robert G Griffin*[2,3], *Susan Lindquist*[1,4,5]. 1) Whitehead Institute, Cambridge, MA; 2) Department of Chemistry, MIT, Cambridge, MA; 3) Francis Bitter Magnet Laboratory, MIT, Cambridge, MA; 4) Howard Hughes Medical Institute, Cambridge, MA; 5) Department of Biology, MIT, Cambridge, MA.
Almost all biology happens within the boundary of a cell, which is highly crowded and contains a large number of macromolecules with the potential to interact. Yet structural investigations of biomolecules are typically confined to

simplified in vitro systems. While nuclear magnetic resonance (NMR) spectroscopy yields atomic-level information and has no requirement for crystalline samples, it often requires tens of milligrams of isotopically enriched material. For this reason, the theoretical ability to investigate low-concentration isotopically labeled biomolecules within unlabeled complex biological mixtures is usually not possible due to impedingly long acquisition times. Dynamic nuclear polarization (DNP) is able to dramatically increase the sensitivity of NMR, making studies of low-concentration samples in complex environments experimentally tractable. Yeast prions are self-templating protein-based mechanisms of inheritance whose conformational changes lead to the acquisition of diverse new phenotypes. The best studied of these is the yeast prion protein Sup35. Different regions of the Sup35 protein are responsible for prion templating, prion inheritance and translation termination. We sought to determine if the structure of Sup35 fibrils formed in the presence of potential organizing protein components such as chaperones differed from those formed in vitro. Using DNP NMR, we find that the N-terminal prion templating domain of Sup35 assembled in the presence of cell lysates was similar to that of purified fibrils while the middle domain involved in prion inheritance experienced a very different chemical environment. This suggests cellular organization of fibrils in vivo is mediated by specific interactions between cellular components and the middle domain, which is intrinsically disordered in purified fibril samples. Thus, the often-observed flexible regions of amyloid fibrils may in fact be intimately involved with cellular components to create a self-organizing mechanism that coordinates fiber deposition. The ability to obtain specific, atomic level insights about a protein at endogenous levels in complex biological milieu may bring unprecedented insights into the effects of differing genetic background on biological structure.

61. High level of chromosomal mosaicism in supposedly clonal yeast cell populations. ***Alexandre Gillet-Markowska***, *Gilles Fischer.* UPMC-CNRS, Paris, Ile-de-france, France.
It is now well established that large Structural Variations (SV) of chromosomes, including duplications, deletions and translocations of large DNA segments, are major contributors to genome polymorphism between individuals. New approaches to single-cell genome dynamics have also recently revealed the quantitative importance of somatic SV within individuals and provided strong evidence that genomes would be much more plastic than previously thought. However, a large number of structural variants probably remain out of reach either because they are present at a too low frequency in the population or because their breakpoints involve large dispersed repeated sequences. We developed a highly innovative approach aiming at characterizing all type of chromosomal rearrangements, at the genome scale, at the time they appear in individual cells from large populations of *Saccharomyces cerevisiae*. This method is experimentally relatively straightforward as it relies on a simple DNA extraction from cell culture followed by deep coverage long-range paired sequencing of large DNA inserts. We developed a dedicated algorithm, Ulysses, that can accurately detect all types of SVs even those that are present in a small proportion of the population through a scoring procedure based on the estimation of the statistical significance of each candidate SV. Experimental validation through PCR amplification of rare rearrangement junctions demonstrated the existence of frequent structural variations within supposedly clonal yeast populations grown in the absence of selection, demonstrating that clonal populations in fact represent heterogeneous mosaics comprising cell lines carrying various SVs.

62. Structured metabolic response to cellular stress. ***Adam Rosebrock***[1], *Julia Hanchard*[1,2], *Olga Zaslaver*[1,2], *Chris Go*[1,2], *Ying Zhang*[1,2], *Amy Caudy*[1,2]. 1) Donnelly Centre, University of Toronto, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.
Birds of a feather flock together; related biological signals do, too. Analyzing perturbed states of a complex system can yield powerful functional insights. We have used mass spectrometry metabolomics to directly measure the biochemical state of yeast responding to environmental and nutritional stresses, building a compendium of more than 600 metabolite profiles across dozens of conditions. We find that unsupervised clustering of metabolite abundances across a range of conditions is sufficient to reconstruct known biosynthetic pathways, identify previously unknown metabolic side-products, and identify intermediates in known and previously uncharacterized metabolic reactions.
Our experimental conditions intentionally parallel the landmark gene expression analysis of stress response performed by Gasch and colleagues nearly 15 years ago. This design has enabled us to compare regulation of metabolism and expression at a systems level. We find that, in contrast to the concerted general stress response observed in transcriptional studies, metabolic response to stress is highly structured and reflects a large number of condition-specific biochemical changes. Generating our large primary data set required development of tools for comparison of metabolite levels across diverse samples and many experiments. In addition highlighting our discoveries of condition-specific metabolic response, I will discuss how we harnessed the awesome power of yeast metabolism to elevate small molecule mass spectrometry from biochemical stamp collection to a robust, quantitative platform for systems analysis of cellular state.

63. The use of fluorescence cross-correlation spectroscopy to assay information about protein complexes in yeast. *Brian Slaughter, Jay Unruh, Christine Smoyer,* ***Sue Jasperson***. Stowers Institute for Medical Research, Kansas City, MO.
Understanding spatial and temporal dynamics of protein-protein interactions inside the cell is important to virtually every

aspect of biology. Yeast has served as a pioneer for the development of a variety of techniques used to address this problem, including the yeast two-hybrid system, the bimolecular complementation assay, a myriad of reporter systems, affinity tags for co-immunoprecipitation and fluorescence resonance energy transfer (FRET). Recently, we have fine-tuned line scanning fluorescence cross-correlation spectroscopy (FCCS) assays to measure protein complex formation in the nuclear membrane in budding yeast. In contrast to FRET, FCCS is not restricted to immediate physical interactions (though it does require co-diffusion), and is amenable to live cells, as opposed to chemical cross-linking. The principle behind FCCS is simple: if labeled proteins are present in a complex, they will synchronously migrate through the focal volume; if the proteins do not associate, their migration will be random. Quantitative information about the number of particles and their rate of diffusion within the focal volume can be determined using correlation analysis. FCCS can be done on endogenously expressed proteins and is well suited to membrane proteins, which account for ~10% of all proteins present in the yeast proteome. We provide examples of its use on the nuclear envelope, ER and other intracellular membranes and discuss how a related method can be used to study spatial and temporal formation of soluble protein complexes.

64. Quantifying the functional impact of all possible missense variants of BRCA1. ***Lea Starita***[1], *Jacob Kitzman*[1], *Justin Gullingsrud*[1], *Jeffrey Parvin*[3], *Jay Shendure*[1], *Stanley Fields*[1,2]. 1) Genome Sciences, University of Washington, Seattle, WA; 2) HHMI; 3) The Ohio State University, Columbus, OH.
BRCA1 is a breast and ovarian cancer-specific tumor suppressor protein that binds to the BARD1 protein to act in double-strand DNA break repair via homologous recombination. Sequencing of BRCA1 has identified both known cancer-predisposing mutations as well as nearly 400 missense mutations classified as variants of uncertain significance (VUS). Test results reported as VUS make decisions about cancer screening and prevention difficult and stressful for women with a family history of breast cancer, as there are no consistent clinical guidelines for advising them. We have developed massively parallel assays in yeast, in phage and in vitro to score all 35,397 possible single amino acid substitutions in BRCA1 for their effects on biochemical and cellular functions. To date, we have examined the N-terminal 304 amino acids - the BRCA1 RING domain - and scored 4,600 of the possible 6,060 variants (75%) in both a phage display assay that quantifies ubiquitin ligase activity and a yeast two-hybrid assay that scores binding to BARD1. The scores for variants in both assays reiterate the functional importance of the 4-helix bundle shared between BRCA1 and BARD1 and the zinc-coordinating residues of BRCA1. In addition, our results reveal that ubiquitin ligase activity does not perfectly correlate with cancer predisposition, and that many cancer-predisposing variants have lost ligase activity because they fail to bind to BARD1. We identified several BRCA1 variants classified as VUS that are also nonfunctional ligases and that fail to bind to BARD1. On the other hand, we identified variants that have been hypothesized to be pathogenic by Grantham chemical difference scores, but have wild-type function. These two assays as well as others provide data that should be useful in assessing cancer risk for those with BRCA1 VUS status.

65. Using DNA Repair Mutants for Cancer Drug Discovery and Identifying Chemoresistance Targets. *Irene Ojini*, ***Alison Gammie***. Princeton University, Princeton, NJ.
Nearly 20% of all colorectal cancers are caused by deficiencies in DNA mismatch repair, a highly conserved mechanism required for genomic stability. Loss of mismatch repair also plays a role in the development of chemoresistance, a primary challenge in medical treatments for conditions ranging from cystic fibrosis to cancer. Chemical synthetic lethality is an emerging strategy for specifically targeting cells with cancer-driving mutations. Because mismatch repair deficient tumors accumulate mutations at a high rate and become heterogeneous, targeting the variety of potential pathways altered in these cancer cells is challenging. Therefore, targeting the primary vulnerability is a rational approach for a broad-spectrum treatment. We use mismatch repair deficient yeast strains in synthetic lethality and drug resistance chemical screens with the goal of discovering novel and effective treatment regimes. In a pilot screen using over 2,500 compounds from the NIH sponsored Open Chemical Repository we identified 29 compounds that cause a differential growth when comparing the wild-type and mismatch repair null strains. Most resulted in resistance; however, synthetic growth defects were observed for two of these compounds. As an extension of this screen we exploited the fact that mismatch repair defective cells are mutators to identify drug resistance mutations using whole genome sequencing. The utility of this approach was validated with the identification of *CAN1* and *TOP1* drug resistance targets for two compounds, canavanine and camptothecin, respectively. We find that this method is useful in the identification of a range of single gene and polygenic resistance targets. Finally, we provide evidence that the mechanistic insights gleaned from these experiments allow for the development of combination therapeutic regimes to simultaneously target cells that confer resistance during a primary treatment to prevent chemoresistance from arising. In summary, the rapid and robust nature of this technique can accelerate the identification of drug resistance targets and guide the development of novel therapeutic combination strategies. Our inexpensive approach could also serve as a foundation for future cell-based DNA repair mutant assay as an anticancer drug and drug resistance discovery platform.

66. Towards humanizing budding yeast. ***Aashiq H. Kachroo****, Jon M. Laurent, Christopher Yellman, Edward M. Marcotte.* Center for Systems and Synthetic Biology, The University of Texas at Austin, Austin, TX.
Genes essential for cell growth are often conserved through evolution, with orthologous genes performing similar functions even in dissimilar organisms. As genes in different lineages can diverge relative to their shared ancestors, it is not a foregone conclusion that, for example, a yeast gene performs exactly the same functions as its human ortholog, given the 1 billion years of accumulated mutations since the yeast and human lineages diverged. Nonetheless, some human genes can replace their yeast orthologs, i.e. their DNA and encoded proteins can substitute for the yeasts own copies, confirming that the extant genes perform the critical functions of the common ancestor gene in the cellular context of both yeast and humans. However, it is unknown if replaceability is a general property of distant orthologs. Yeast have 547 essential genes with single, well-defined (1:1) human orthologs, so in order to better understand factors governing replaceability, we systematically humanized these genes, testing if the human genes could replace their yeast counterparts. Many (~47%) of the genes could be humanized with minimal effects on yeast cell growth. Orthologs sequence similarities and abundances only partly predict replaceablity. Notably, replaceability is a modular property: genes encoding proteins within the same complex or pathway tend together to be either replaceable (e.g., the proteasome) or not (e.g., DNA replication). Our data suggest that critical functions of many essential genes are retained intact through evolution, largely unaffected by splicing or codon alterations, and not subject to excessive neutral drift in their key ancestral functionalities.

67. Organelle deterioration with age: The limits of an interconnected cellular system. ***Dan Gottschling***. FHCRC - Seattle, WA.
Our approach to studying aging considers the process in light of a fundamental property of biological systems - interconnectivity. All levels of interaction contribute to the ultimate phenotype of an organism: interactions between tissues, cells, organelles, metabolic pathways, genes, and individual molecules. Considering these interactions as a network helps develop new ideas and hypotheses about the aging process. For instance, if we examine a network of interactions within a cell and consider each organelle as a subsystem within the network, then what happens to the other connected organelles when one becomes dysfunctional with age? If a subsystem decays with age and does indeed affect a connected organelle, which interactions are required for this to occur? This simple idea focuses upon connections that create interdependency between two subsystems - e.g. one organelle produces a molecule required for the proper function of another organelle. However, in considering a network of interactions it is likely that more than one subsystem is sensitive to aging, possibly through distinct routes. Taking advantage of a tool we developed, the Mother Enrichment Program (Lindstrom & Gottschling, 2009) that allows us to isolate and examine large populations of synchronously aged S. cerevisiae cells, we have begun to address these questions at the cellular level. To this end we have identified several cell biological subsystems that experience an age-associated decline. For example, we identified an age-associated mitochondrial change that is conserved throughout eukaryotes. We discovered a series of causal events that lead to this change and also impact overall life span of the cell. The earliest step in the aging process that we have defined so far is a reduction in vacuole acidity with age, which in turn leads to a loss of mitochondrial membrane potential (Hughes & Gottschling, 2012). The inability of the vacuole to store amino acids when vacuolar pH increases is responsible for the loss of mitochondrial membrane potential, though the molecular details of this link remain an area of investigation. Loss of mitochondrial membrane potential in turn causes dysfunction of a number of mitochondrial processes. One of note is reduced biosynthesis of iron-sulfur complexes (ISC), an essential cofactor in a number of enzymes, including those involved in DNA replication and repair. In fact, we find that reduced ISC levels during mitochondrial dysfunction lead to nuclear genome instability - a relatively late age-associated phenotype (Veatch et al. 2009, McMurray & Gottschling, 2003). By approaching the study of aging through the lens of interconnectivity, we have identified several linked causal events in the aging process. I will discuss our most recent advances on these fronts.

68. Orphan Diseases: Identifying Genes and NovelTherapeutics to Enhance Treatment (IGNITE). ***Christopher McMaster***. Dalhousie University, Halifax, NS, Canada.
One in twelve Canadians has an orphan disease, defined as a disease found in less than 1 in 2000 persons. Most orphan diseases are inherited monogenic disorders. Of the ~7,000 orphan diseases, 90% have no treatment, 90% are life-limiting, and 50% affect children. IGNITE is a team of clinicians and scientists with the goal of discovering mutations in genes that cause orphan disease, and identifying therapeutic targets and therapies for these same diseases. I will describe the gene discovery and therapy efforts of the IGNITE team as a whole, and then focus on progress toward a therapeutic for an inherited sideroblastic anemia.

69. Chromosome instability and synthetic cytotoxicity in yeast and cancer. ***Philip A. Hieter****, Derek van Pel, Hunter Li, Noushin Moshgabadi, Melanie Bailey, Nigel O'Neil.* Michael Smith Lab, Univ British Columbia, Vancouver, BC, Canada.
Genes that maintain chromosome stability (CIN genes) are conserved in eukaryotes and are often somatically mutated in cancer. We have been identifying synthetic lethal partner genes, in yeast synthetic lethal (SL) interaction networks, that are highly connected with sets of CIN genes somatically mutated in cancer. This identifies hub proteins and processes that are

candidate targets for synthetic lethal killing of cancer cells with defined CIN gene somatic mutations. The protein product of FEN1 (encoding flap endonuclease) was used as a target for small-molecule inhibitor screening using a fluorescence-based assay for enzyme activity. Inhibitors of FEN1 activity in vitro were shown to selectively inhibit the proliferation of cultured cancer cells carrying inactivating mutations in CDC4, or knockdown or inhibition of MRE11A, two genes frequently mutated in a variety of cancers. Model organisms have also allowed us to analyze genetic networks underlying the response to DNA damaging therapeutics to identify candidate clinical applications. DNA damaging agents, such as camptothecin, a topoisomerase I inhibitor that causes DNA damage, are powerful therapeutic tools that can differentially kill cells with an impaired DNA damage response. The response to DNA damage is complex and comprised of a network of coordinated pathways, often with a degree of redundancy. Tumor-specific somatic mutations in DNA damage response genes could be exploited by inhibiting the function of a second gene product to increase the sensitivity of tumor cells to a sub-lethal concentration of a DNA damaging therapeutic agent, resulting in selective killing we have termed Synthetic Cytotoxicity. We used the yeast non-essential gene deletion collection to screen for synthetic cytotoxic interactions with camptothecin and a null mutation in TEL1, the yeast orthologue of the mammalian tumor suppressor gene, ATM. We found and validated 14 synthetic cytotoxic interactions that define at least five functional groups. We found that at least one synthetic cytotoxic interaction was conserved in the nematode worm, Caenorhabditis elegans. This raises the potential for detecting candidate combination therapies in simple model organisms.

Genomics and Proteomics Sunday Session

Determination of in vivo RNA kinetics using RATE-seq. ***David Gresham***, *Benjamin Neymotin, Rodoniki Athanasiadou.*
Center for Genomics and Systems Biology, Department of Biology, New York University, New York, NY.
The abundance of a transcript is determined by its rate of synthesis and rate of degradation; however, global methods for quantifying RNA abundance cannot distinguish variation in these two processes. We have developed a method called RNA Approach To Equilibrium sequencing (RATE-seq), which uses in vivo metabolic labeling of RNA with 4-thiouracil (4sU) and approach to equilibrium kinetics, to determine RNA degradation and synthesis rates. RATE-seq does not disturb cellular physiology, requires minimal normalization, and can be readily adapted for studies in most organisms. We demonstrate the use of RATE-seq to estimate genome-wide kinetic parameters for coding and non-coding transcripts in Saccharomyces cerevisiae. We are applying RATE-seq to study the regulatory role of mRNA degradation in remodeling of the transcriptome in response to extracellular conditions.

Proteomic approach to predicting regulators of yeast response to 4MCHM. Xiaoqing Rong[1], Lihua Jiang[2], Casey Nassif[1], Michael Snyder[2], **Jen Gallagher[1]**
Environmental stresses are constantly changing and yeast have evolved biochemical pathways including multiple drug resistance (MDR) to tolerate a broad class of toxic chemicals. However, there is considerable variation in growth inhibition across genetically distinct yeast strains in response to different chemicals. Although these yeast are sequenced we are facing the challenge of predicting phenotypes from genotypes. Here we describe yeast as a model to understand genetic variation of chemical response. On January 9, 2014 an estimated 10,000 gallons of crude 4-methylcyclohexane methanol (crude 4MCHM) was discovered spilling into the Elk River in Charleston, West Virginia, contaminating tap water for over 300,000 residents. To understand how 4MCHM exposure affects human health we investigated by how 4MCHM affects yeast. We developed a novel method to identify proteins that regulate the response to 4MCHM based on global proteomics of yeast. Two strains showed a wide range of sensitivity to 4MCHM, S288c (a laboratory strain) and YJM789 (a clinical isolate) only when grown in YPD (rich media) but not YM (minimal media). To identify protein(s) that regulate response to 4MCHM, we compared the proteomes of cells grown in rich and minimal media and importantly without 4MCHM present. The proteome of genetically distinct cells vary even when grown in the identical conditions. We identified several candidate proteins that were differentially expressed in YPD but not YM. Med15/Gal11 was required for tolerance to 4MCHM and as part of the mediator complex is known to regulate MDR via interaction with the transcription factors, Pdr1 and Pdr3. The different alleles of Med15 contain expansion of poly glutamine tracts that have been shown in other proteins to induce protein aggregation. Variation across genomes is expected but variation in key proteins are potent modulators of genetic diversity in response to environmental stresses. These proteins are called master variators. Global transcriptomes of the same cells indicate that amino acid and phospholipid metabolism is altered. Growth inhibition by 4MCHM can be suppressed by supplementation of intermediates of the glutathione pathway, which further supports that 4MCHM treatment altering cellular metabolism. We have used proteomics data from unchallenged yeast to uncover changes in protein expression and to predict Med15 as an important regulator in response to 4MCHM.

Profiling the RNA maturation landscape in yeast**.** Alexander Ratushny[1, 2], Marlene Oeffinger[4], Wei-Ming Chen[2], Karen Wei[4], Peter Fridy[3], Richard Rogers[2], Ramsey Saleem[1,2], Garrett Poshusta[1], Michael Rout[3], **John Aitchison**[1,2]1) Systems Biology, Institute for Systems Biology, Seattle, WA, USA.; 2) Seattle Biomedical Research Institute, Seattle, WA, USA; 3) Rockefeller University, New York, NY, USA; 4) Institut de recherches cliniques de Montréal, Montréal, Québec,

Canada All RNAs are transcribed and assembled into ribonucleoprotein (RNP) complexes and these diverse and dynamic complexes play crucial roles in RNA transcription, processing, nucleocytoplasmic export, translation, and decay. While many RNP components are known, given the vast amount of RNA Pol II transcribed RNAs alone, and the relatively small number of known RNA maturation factors, several open questions remain: How much overlap between subsets of proteins exists for different Pol II transcripts? Are all mRNAs processed along the same pathway, or are there distinctions for different classes of transcripts? What is the intra-complex landscape, the direct interactions, within these RNPs? To obtain a comprehensive view on mRNP maturation, we performed a quantitative analysis of yeast RNP complexes at a systems level. In this study, thirty five proteins from different stages of the mRNA maturation pathway (from transcription through different processing steps to export and degradation of faulty complexes) were selected for analysis. The RNP complexes of the selected protein A-tagged proteins have been isolated under different conditions and from different genetic backgrounds (i.e., conditional and deletion mutants). The bait-associated RNAs and proteins were isolated by single-step affinity purification and analyzed by RNA-seq and mass spectrometry, respectively. This systems biology approach detects many thousands of high quality and reproducible protein-protein and protein-RNA interactions as well as complex network rewiring events of multiple targeted RNP composites under conditional perturbations. The systematic functional and network analysis of this unprecedented data set reveals hundreds of previously uncharacterized protein-protein and protein-RNA interactions and novel molecular control mechanisms of the coordination of cellular processes that are now experimentally validated in a targeted manner.

A Chemical-Genetic Matrix Strategy for Directed Discovery of Small Molecule Synergizers. Jan Wildenhain[1], Sonam Dolma[2], Michaela Spitzer[1,3], Gerry Wright[3] and **Mike Tyers**[1,4] [1]University of Edinburgh, Edinburgh EH9 3JR United Kingdom; [2]The Hospital for Sick Children, Toronto, M5G 1X8 Canada; [3]McMaster University, Hamilton L8N 3Z5 Canada; [4]University of Montreal, Montreal, Québec H3C 3J7, Canada
Cell function is governed by a complex network of genetic interactions, such that combinations of genetic mutations can either exacerbate or ameliorate any given phenotype. Predicated on this network concept, we have combined sensitized genetic screens, interaction network data and cheminformatics to discover combinations of compounds that exhibit synergistic bioactivities. We built a comprehensive Chemical Genetic Matrix (CGM) of chemical-genetic interactions using the budding yeast Saccharomyces cerevisiae as a model system. A query set of 3,672 small molecules was used to probe 195 different deletion strains for genotype-specific inhibition of cell growth. This CGM dataset was combined with chemical structure information using a Laplacian modified Naïve Bayes multi-class learner to uncover potential chemical-genetic relationships within the data. The CGM data was then integrated with extensive genetic interaction datasets to build a second order network activity response (SONAR) graph to identify chemical combinations that mimic genetic loss-of-function phenotypes. Of 896 molecules that showed specific bioactivity across deletion strains, we selected 128 compounds and tested all possible combinations to generate a gold-standard dataset for validation. We verified our predictive approach on the resulting 8128 drug combinations and were able to predict synergy with high accuracy. We discovered 58 previously unidentified small molecule combinations for which synergism was verified in dose-response surfaces. 10 of these combinations were tested against a spectrum of pathogenic fungal species, including C. albicans, C. parapsilosis, C. neoformans and C. gattii. Some combinations exhibited species-specific toxicity, suggesting that it will be possible to design highly selective drug combinations that target pathogenic species but not benign commensal organisms. The CGM approach can cross-connect genetic pathways through chemical space, identify novel antifungal agent combinations, and serve as a resource for small molecule probes across many areas of cell biology.

Mapping the cellular response to small molecules using chemogenomic fitness signatures. **Guri Giaver**.
Genome-wide characterization of the in vivo cellular response to perturbation is fundamental to understanding how cells survive stress. Identifying the proteins and pathways perturbed by small molecules affects biology and medicine by revealing the mechanisms of drug action. We used a yeast chemogenomics platform that quantifies the requirement for each gene for resistance to a compound in vivo to profile 3250 small molecules in a systematic and unbiased manner. We identified 317 compounds that specifically perturb the function of 121 genes and characterized the mechanism of specific compounds. Global analysis revealed that the cellular response to small molecules is limited and described by a network of 45 major chemogenomic signatures. Our results provide a resource for the discovery of functional interactions among genes, chemicals, and biological processes.

Improving the yeast metabolic model using LOPIT proteomic data. **Stephen G Oliver**, Yuchong Wang, Duygu Dikicioglu, and Kathryn S LilleyCambridge Systems Biology Centre & Dept. Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK
The metabolic model of the yeast Saccharomyces cerevisiae is the most complete for any eukaryotic organism. At a coarse-grained level, the model successfully predicts the overall topology of the network of interactions between metabolic genes. However, at the single-gene level, it only predicts a small fraction of the interactions revealed by synthetic genetic array (SGA) experiments. Moreover, predictions from the metabolic models for bacteria are much more accurate than are those for yeast. While it is clear that the content and connectivity of the yeast model can be improved, can it really be that

our view of yeast metabolism is so far behind that for bacteria, or is something else missing? Part of the answer may lie in the accurate representation of sub-cellular compartmentation. In an effort to improve this, we have studied the sub-cellular localisation of proteins using the LOPIT (Localisation of Organelle Proteins by Isotope Tagging) approach and an improved cell fractionation protocol. In all, > 1600 proteins have been assigned to eight major subcellular compartments, namely endoplasmic reticulum, Golgi, plasma membrane, cytoplasm, ribosome and proteasome, vacuole, mitochondria, and nuclei. We have used these observations on compartmentation to establish the biological context to improve the ability of metabolic models to predict lethal or synthetically lethal phenotypes.

Extensive diversity in the transcriptional output from the yeast genome. **Lars Steinmetz.**
Genome-wide pervasive transcription has been reported in many eukaryotic organisms, revealing a highly interleaved transcriptome organization that involves hundreds of non-coding RNAs with the potential to condition phenotypes. These include SUTs (Stable Unannotated Transcripts) and CUTs (Cryptic Unstable Transcripts) that we identified in yeast by profiling the transcriptome in multiple conditions, mutants, and strain backgrounds. We have shown that most non-coding RNAs (SUTs and CUTs) initiate from bidirectional transcription of promoters, and that gene looping helps to maintain the directionality of transcription from these promoters. We have also characterized antisense transcripts genome-wide, demonstrating that they play a key role in shutting off gene expression and extending its variability. In addition to complexity that exists across the genome in terms of expressed regions on each strand, there is an extensive hidden layer of complexity found in alternative overlapping transcripts at each individual gene. Recently, we have demonstrated that these alternative overlapping isoforms can result in divergent post-transcriptional regulation.

70A. Understanding the regulation of motor proteins in *cdc15-2* cells recovering from spindle damage. ***Beryl Augustine***, *Foong May Yeong*. Dept of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
The mitotic spindle is a highly dynamic structure assembled during each cell division cycle for facilitating chromosome segregation. Tight regulation of spindle dynamics is crucial for ensuring faithful chromosome segregation not only during normal cell cycle progression but also upon recovery from spindle damage. This depends to a large extent on the timely activities of motor proteins in controlling spindle lengths. For instance, our lab has previously shown that in cdc15-2 (a temperature-sensitive allele of the Hippo-like Kinase) cells, chromosomes are mis-segregated during recovery from spindle assembly checkpoint (SAC) activation due to the abnormal persistence of the Cin8p motor protein on the interpolar microtubules. This led to the premature elongation of the spindle even before kinetochores were properly captured.
Although, the roles played by motor proteins in determining spindle dynamics have been characterized, the regulation of motor proteins themselves during recovery from spindle damage remains relatively-unexplored. In this study we investigated how various key motor proteins are affected during SAC recovery. Using Western blot analysis, we characterized the abundance of the various motor proteins in wild type and *cdc15-2* cells recovering from exposure to Nocodazole (Noc). From time-lapsed fluorescence microscopy, we studied the dynamic localization of different motor proteins relative to chromosome segregation and spindle dynamics in wild type and *cdc15-2* cells released from Noc-treatment. We present data showing sequential disappearance of motor protein signals from the midzone or spindles in wild-type cells and provide a comparison of the order of removal to that in *cdc15-2* cells. Our results indicated that the localization and subsequent loss of motor proteins might be regulated in a spatio-temporal manner that is affected in the absence of proper Cdc15p function.
Taken together our data provide a deeper understanding of how the dynamics of spindle recovery after damage depends upon a tight control over the abundance and localization of motor proteins.

71B. Physiological impact of NAD(P)HX accumulation in yeast. ***Julia Becker-Kettern***, *Paul P Jung, Carole L Linster.* Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg.
One of the revelations of the genomics revolution is that for most organisms the majority of encoded proteins remain without an identified function. Many of those unknown proteins are members of widely conserved families, and significant proportions are probably enzymes. Accumulating evidence suggests that many enzymes of unknown function are involved in a secondary metabolism that remains largely absent from the metabolic reconstructions used to generate genome-scale models of metabolism. This secondary metabolism can comprise of reactions that generate, eliminate or repair damaged small molecules that are useless at best and toxic at worst. The term metabolite repair has been coined to describe this process. These abnormal metabolites can be generated by unwanted chemical reactions or by enzymatic side reactions. Examples for such damaged metabolites are NADHX and NADPHX, hydrated forms of NADH and NADPH, respectively, which are both inactive as cofactors. Two enzymes that specifically repair these molecules have only recently been biochemically characterized: NAD(P)HX epimerase and NAD(P)HX dehydratase (YNL200c and YKL151c, respectively). However, the physiological relevance of the two enzymes still remains largely unclear. To address this, yeast knock-out strains lacking the NAD(P)HX repair enzymes were generated. Using HPLC and LC-MS methods we

were able to prove intracellular accumulation of NAD(P)HX in the knock-out strains at certain growth stages. In accordance with previously published *in vitro* observations, the intracellular NADHX accumulation could be enhanced further when cells were exposed to higher temperatures or engineered to overexpress *TDH1* or *TDH2*, two isoforms of the glycolytic enzyme GAPDH in yeast. Increased NAD(P)HX levels were accompanied by a decrease in intracellular NAD^+-levels, leading to the assumption that NADHX repair deficiency might affect the life span of cells. Therefore, we are currently investigating both replicative and chronological aging. Preliminary results support a decrease in chronological life span under certain conditions. Replicative life span assays are ongoing. In addition, non-targeted metabolomics analyses and RNA tiling arrays have been performed to uncover other cellular reactions and processes that might be affected by intracellular NAD(P)HX accumulation.

72C. A cell separation checkpoint ensures proper order of late cytokinetic events. ***Jennifer L. Brace***, *Matthew Doerfler, Eric L. Weiss.* Molecular Biosciences, Northwestern Univ, Evanston, IL.
Checkpoint pathways have evolved to ensure that critical steps of cell replication and division are completed accurately and in the proper sequence. Physical separation of cells by cytokinesis and abscission exemplifies precise coordination of diverse processes in time and space, but the mechanisms that enforce this order are incompletely understood. In budding yeast cytokinesis, a chitin-rich septum is formed coincidently with plasma membrane ingression and contraction of an actomyosin ring. Minutes later, the completed septum is destroyed by secretion of a cohort of cell separation enzymes that includes the chitinase Cts1, leading to cell separation. While transcription of cell separation genes is tightly linked to mitotic exit by a hippo signaling pathway called the RAM network, the expression of these genes is induced just prior to cytokinesis. How, then, do cells avoid destroying the septum before it is finished? Our findings suggest that a checkpoint-like pathway is activated when septum formation is disrupted, preventing premature degradation of the forming structure. We find that cytokinetic mutants, which do not form normal septa, produce the chitinase Cts1 with normal timing but do not secrete it. This supports a model in which Cts1 secretion may be regulated by a checkpoint. Further indicating that proper control of the cell separation program is critical when septum formation is delayed, cytokinetic mutants are suppressed by deletion of *CTS1* and are highly sensitive to *CTS1* overproduction. We have also found that the RAM network, which turns on the cell separation program, may play a role in ensuring its proper timing. A new *in vivo* substrate of Cbk1, a key RAM network Ndr/LATS kinase, exhibits strong genetic interaction with cytokinetic mutants, and this is suppressed by a deletion of *CTS1*. Overall, these data support the existence of a checkpoint-like mechanism that monitors completion of the septum and prevents secretion of enzymes that destroy it, ensuring the proper timing of cell separation events.

73A. Respiro-fermentative differentiation in yeast colonies. ***Michal Cap***[1], *Libuse Vachova*[2], *Zdena Palkova*[1]. 1) Department of Genetics and Microbiology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic; 2) Institute of Microbiology of the ASCR, v.v.i., 142 20 Prague 4, Czech Republic.
Unicellular microorganisms grown on solid surfaces form multicellular structures - colonies and biofilms. Many studies have revealed unanticipated complexity within these structures including cellular differentiation giving rise to multiple cell-types differing in their morphology, physiology and metabolism. We have previously shown that this differentiation occurs also in aging colonies of Saccharomyces cerevisiae laboratory strains growing on non-fermentative carbon source. Two prominent layers are clearly differentiated in aging, two-week old colonies: the upper layer is formed of larger cells (U cells) exhibiting longevity, stress resistance and metabolically active status, while lower cells (L cells) are smaller, stress sensitive and show signatures of starvation (Cap et al, Mol Cell, 2012). Both populations are non-dividing and represent different aging populations. Here, we present differences found in basic carbon metabolism and energetic status of the two cell types. On the levels of transcript differences, enzymatic activities and cellular metabolism, U cells display higher activity of glycolytic enzymes and higher fermentative capacity, while L cells have higher activity of TCA enzymes and respiratory capacity. Thus, U cells exhibit fermentative or respiro-fermentative metabolism and L cells relay on respiratory metabolism. Energetic status of the two cell types also differ: U cells accumulate more glycogen and lipid droplets and exhibit higher ATP energy charge and NADH/NAD+ ratio than L cells, confirming the metabolically more active status of U cells and starvation in L cells. The project is co-financed by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

74B. Cell Cycle Regulation of Endocytic Vesicle Scission Apparatus during Mitotic Exit in *Saccharomyces cerevisiae*. *Kaiquan Tan*, ***Cheen Fei Chin***, *Foong May Yeong.* Biochemistry, National University of Singapore, Singapore.
Clathrin-Mediated Endocytosis (CME) is the major route for protein cargo internalization from the plasma membrane. In budding yeast, CME are known to be active throughout the cell division cycle. Indeed, our Western blot analysis of key CME components including proteins of the vesicle scission apparatus (VSA), Rvs161p, Rvs167p and Vps1p, revealed that they were ubiquitously expressed throughout the cell division cycle. Previous studies on the localization of key CME machineries showed these components to be largely found in the cytoplasm and plasma membrane. However, the spatio-temporal regulation of these proteins specifically at the division site during mitotic exit has not been previously

demonstrated. In this study, we identified association among Rvs161p, Rvs167p and Vps1p during mitotic exit. We further isolated Gyl1p and Gyp5p as Rvs167p-associated proteins during metaphase and mitotic exit. Using time-lapsed fluorescence microscopy, we characterize the dynamics of VSA localization relative to a mitotic exit marker and endocytic cargo, Chs2p, at the end of mitosis. We observed that the Rvs161p and Rvs167p translocated from the cytoplasm to the neck occurred subsequent to Chs2p neck localization when Cdk1p activity was low. Interestingly, we established the inter-dependence of the VSA components in neck localization. While it has been previously demonstrated that the Rab GTPase Activating Proteins (GAP), Gyl1p and Gyp5p are required for the recruitment of Rvs167p to the small bud-tip during polarized growth, our study provide data showing that Gyl1p and Gyp5p also play a role in regulating the neck localization of Rvs161p and Rvs167p during mitotic exit. Moreover, endocytic cargo internalization was defective in the absence of *RVS161* and *RVS167*. Taken together, our data indicated that the timely accumulation of CME components at the neck in late mitosis is needed for the internalization of cytokinesis enzymes and membranes during septation and cytokinesis.

75C. The metabolic response to acetic acid stress in *Saccharomyces cerevisiae*. ***Yachen Dong***, *Zhihua Jiao, Jin Cai, Ruosi Fang, Qihe Chen.* Department of Food Science and Nutrition, Zhejiang University, Hangzhou, China.
Acetic acid is a toxic inhibitory compound in alcoholic fermentation, while it can trigger programmed cell death (PCD) in both yeast and mammalian cells. Understanding the response processes in *Saccharomyces cerevisiae* to acetic acid stress is highly desirable for the production of fine and bulk chemicals, but also for biomedical research. The phenotypic properties and physiological scenarios of PCD induced by acetic acid have been widely reported, but the metabolic response to acetic acid stress still needs to be elucidated. Thus, we investigated the metabolite profiling at different stages in acetic acid-induced PCD based on metabolomics. In this work, the yeast cells exposed to acetic acid were assayed by different apoptotic markers at different times. Metabolic analysis revealed that metabolites involved in carbohydrate metabolic pathways were sensitive to acetic acid, varying over times. Among the metabolites measured, part of amino acids, organic acids and phosphates were also detected to respond strongly to the acetic acid stress. The metabolomic results advanced the molecular events underlying the response to acetic acid, providing a wealth of information on metabolic flux changes mediated by acetic acid for its control and application both in metabolic engineering and biomedicine.

76A. A genetic selection to identify new components of the *S. cerevisiae* RAM network. ***S. Edwards***, *J. Jansen, J. Brace, E. Weiss.* Molecular Biosciences, Northwestern University, Evanston, IL.
The *Regulation of Ace2 and Morphogenesis* (RAM) network of *S. cerevisiae* is a signaling system related to metazoan hippo pathways that plays critical roles in polarized growth and the separation of mother and daughter cells. In addition to Ace2, which is a regulatory target of the RAM network, six components of this pathway have been identified: Cbk1, Kic1, Mob2, Tao3, Hym1, and Sog2. We hypothesize that additional components are required for the systems function. We have designed a powerful genetic approach to identify mutant alleles that reduce or eliminate RAM network function by developing strains in which *URA3* and *HIS3* genes are under the control of Ace2-driven promoters. After selecting for 5-FOA resistance following UV mutagenesis we identified hundreds of independent strains in which RAM network function is compromised. All of these, however, were alleles of known components of the pathway. To modify this screen to suppress isolation of mutant alleles of known RAM network genes we have recently begun construction of a haploid *S. cerevisiae* strain that has two copies of Ace2 and each of the known RAM network genes. We predict that this genomic configuration will provide a probabilistic shield against the isolation of known network components in the selection described above. The ability to largely avoid isolation of alleles affecting known network components will in turn allow very large-scale mutagenesis without an excess number of undesired mutants. At present, development of the partial diploid strain is nearing completion, with a second copies of most known RAM network alleles already successfully incorporated. We hope to complete strain development and subsequently transition to mutagenesis experiments within the upcoming months.

77B. Quantifying Condition-Dependent Intracellular Protein Levels Enables High-Precision Fitness Estimates. ***Kerry A. Geiler-Samerotte***[1], *Tatsu Hashimoto*[3], *Mike Dion*[4], *Bogdan Budnik*[4], *Edo Airoldi*[3], *D. Allan Drummond*[2]. 1) Center for Genomics and Systems Biology, New York University, New York, NY; 2) Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA; 3) Department of Statistics, Harvard University, Cambridge, MA 02138, USA; 4) FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138, USA.
The close link between growth rate and fitness, coupled with the sensitivity of growth to genetic and environmental perturbations, has made growth rate among the most-studied phenotypes in evolutionary biology. But how cells regulate their growth in response to environmental and internal cues remains unclear, despite broad conservation across eukaryotes of the genes and pathways which regulate growth. Recent studies in budding yeast, Saccharomyces cerevisiae, have identified a substantial repertoire of genes (25% of the genome) whose transcripts and proteins covary with growth rate independently of the nutrient deficiency that limited growth. We probe this growth-rate response on a finer level, using whole-proteome mass spectrometry to study protein-level changes and growth-rate changes an order of magnitude smaller

than in previous studies. We find a set of proteins whose levels, in aggregate, enable prediction of growth rate to a higher precision than direct growth measurements. However, we find little overlap between these proteins and those that closely track growth rate in other studies. Further, we show that, in yeast, growth rate is not inextricably linked to the levels of any set of proteins (not even ribosomal proteins). This unexpected result suggests that the pathways that set the pace of cell division can differ depending on the growth-altering stimulus. Still, perturbation-specific protein measurements can provide high-precision growth estimates that allow extension of phenotypic growth-based assays closer to the limits of evolutionary selection.

78C. Parsing the many possible physiological roles of trehalose in yeast. ***P. Gibney***, *A. Schieler, J. Chen, Y. Xu, J. Rabinowitz, D. Botstein.* Lewis-Sigler Institute, Princeton University, Princeton, NJ.
Many different physiological roles have been attributed to trehalose and/or trehalose-6-phosphate: chemical chaperone, glycolytic regulator, water substitute, storage carbohydrate, cell cycle regulator, and signaling molecule. While these roles are not necessarily mutually exclusive, it is unclear which are causative, which are correlative, or if trehalose-6-phosphate and trehalose act independently. Here I describe classic genetic and genome-scale approaches to dissect direct and indirect mechanisms of trehalose and trehalose-6-phosphate activity.
We have developed a system to experimentally manipulate levels of intracellular trehalose with precise temporal control. This system was engineered into a number of trehalose metabolism mutant strains, and the effect of trehalose on mediating a variety of cellular phenotypes was examined. We show that this experimental system does allow accumulation of intracellular trehalose, as it suppresses the *ath1* strain inability to grow on trehalose as the sole carbon source. Accumulation of intracellular trehalose does not provide thermoprotection to cells lacking *TPS1*, nor does it repair the carbon-source-specific growth or sporulation defects associated with trehalose biosynthesis mutants. This suggests that the trehalose molecule per se may not be the major determinant of these phenotypes. Further, using this system we show that intracellular accumulation of trehalose or maltose has a negative impact on cellular growth rate. These results are a first step in determining the precise role of the trehalose molecule in the cell, separate from the role of the trehalose metabolic pathway.

79A. Global analysis of molecular fluctuations associated with cell cycle progression in *Saccharomyces cerevisiae*. ***B. Grys***[1], *H. Friesen*[1], *O. Kraus*[2], *B.J. Frey*[2], *C. Boone*[1], *B.J. Andrews*[1]. 1) Donnelly Centre for Cellular and Biomolecular Research, Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Electrical and Computer Engineering, University of Toronto, Toronto, Ontario, Canada.
The regulation of protein expression, turnover, and localization has been recognized as imperative for eukaryotic cell cycle progression. However, there has been no systematic study of proteomic fluctuations throughout the cell cycle in eukaryotes. With its genetic tractability, short cell cycle, and available resources for systematic analysis, the budding yeast, *Saccharomyces cerevisiae* represents a powerful model system with which to address this experimental void. We are optimizing an experimental pipeline for tracking changes in protein localization and abundance over the course of the cell cycle in yeast. This pipeline combines Synthetic Genetic Array (SGA) analysis with high-throughput fluorescence microscopy of the Green Fluorescent Protein (GFP) collection to generate image-based data for ~75% of the yeast proteome. Our experimental approach involves introducing fluorescent markers for cell cycle position into the GFP collection which permits the computational classification of yeast cells into one of six predetermined cell cycle stages based on a novel classification framework that we have developed. We have worked to automate cell cycle classification using a supervised neural network-based approach that functions with ~80% accuracy in our preliminary analysis. We are also adapting our neural network classification method for automated assignment of GFP-tagged proteins to one of 19 subcellular compartments. When combined with other data, such as cell cycle transcriptional information, this unique platform will provide a resource of that can be mined to better characterize existing pathways of cell cycle control, while also identifying novel players in the regulation of cell growth and division.

80B. Untargeted metabolomics reveals the rate of secondary mutations causing metabolic phenotypes. ***Julia A. Hanchard***[1,2], *Adam P. Rosebrock*[2], *Amy A. Caudy*[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada.
How often does a metabolic change result from gene disruption? How many spontaneous second-site mutants conferring metabolic phenotypes are lurking in our freezers? Many experiments in systems biology rely on the use of engineered loss of function mutants and necessarily assume that phenotypic changes result from the engineered lesion. However, spontaneous secondary mutations may also result in phenotypic changes. As part of our ongoing efforts to discover new enzymes, we measured metabolic phenotypes resulting from loss of function mutants for more than one hundred candidate enzyme uncharacterized yeast ORFs taken from our recently published prototrophic version of the yeast deletion collection. To our surprise, we identified metabolic phenotypes unlinked to the loss-of-function mutant.
To quantify the frequency of metabolic phenotypes caused by unlinked mutations, we backcrossed each mutant and

measured metabolite levels by full-scan untargeted metabolomics on multiple independent spores. We determined the metabolic phenotypes that result from engineered deletions and estimated the proportion due to spontaneous secondary mutations. Striking unlinked metabolic phenotypes were seen; we have used classical mapping techniques, empowered by mass spectrometry, to map a subset of these mutants. One mutant conferred a genetically simple 100-fold increase in mevalonate levels. We identified a single point mutation in ERG12 (G392D), mevalonate kinase, as the causal lesion. This mutated residue maps closely to a human mutation that gives rise to HIDS, an inborn error of metabolism which involves mevalonate kinase deficiency leading to mevalonate accumulation. The discovery of a hypomorphic allele of mevalonate kinase is an example of secondary mutations giving rise to metabolic phenotypes.

81C. Functions of genes typical of structured colony morphology. ***Otakar Hlavacek***[1], *Vratislav Stovicek*[2], *Libuse Vachova*[1], *Zdena Palkova*[2]. 1) Laboratory of Cell Biology, Institute of Microbiology, ASCR, v.v.i., 142 20 Prague 4, Czech Republic; 2) Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, 128 44 Prague 2, Czech Republic.
Phenotypic switching is a process commonly observed in pathogenic as well as non-pathogenic yeast and it is defined as a random event, the frequency of which can, however, be affected by environmental conditions. By phenotypic switching yeast can increase their ability to survive under variety of changing external (often harmful) conditions. An example of phenotypic switching is a domestication of wild Saccharomyces cerevisiae strains forming structured colonies, leading to formation of clones that start to form smooth colonies resembling colonies of laboratory strains [1]. In addition, domesticated strains can perform reversible phenotypic switching to feral strains, forming again structured colonies. By comparing of transcriptomes of colonies formed by the three related strains (wild versus domesticated versus feral strains) we identified genes, the expression of which is typical for structured colony life style [2], that is accompanied by presence of various defense mechanisms [3]. The main functional groups of genes induced in structured colony morphotype are genes linked to cell wall remodeling and plasma membrane characteristics as well as genes involved in signaling cascades. The effects of deletions of particular genes on colony morphology and cell properties will be discussed. This work was supported by GACR 13-08605S and the Ministry of Education, Youth and Sports of the Czech Republic project CZ.1.07/2.3.00/20.0055 from the European Regional Development Fund in the Czech Republic. [1] Kuthan et al. (2003) Mol Microbiol 47:745-54. [2] Stovicek et al (2014) BMC Genomics 15:136. doi: 10.1186/1471-2164-15-136. [3] Vachova et. al (2011) J Cell Biol 194: 679-87.

82A. Investigating the role of CDK-mediated phosphorylation of the bud neck-localized proteins Bud3 and Bni4. ***Jennifer K. Hood-DeGrenier***, *Bharat Hans, Angeline Cloutier, Alyssa Geddis, Hanh Nguyen.* Biology Department, Worcester State University, Worcester, MA.
Mass spectrometry-based screens identified the bud-neck localized *S. cerevisiae* proteins Bud3 and Bni4 as cytoplasmic targets of the mitotic cyclin-dependent kinase (CDK) Clb2/Cdc28. Bud3 is critical for the axial bud site selection pattern of haploid yeast cells, while Bni4 is required for targeting the protein phosphatase Glc7 and chitin synthase III (Chs3) to the bud neck and is important for chitin ring assembly, septum formation, and bud neck integrity. To investigate the role of CDK-mediated phosphorylation of Bud3, we mutagenized three consensus phosphorylation sites to alanine (to render them non-phosphorylatable) and to either glutamate or aspartate (to mimic phosphorylation), both individually and in combinations. Both types of mutations reduced the percentage of cells exhibiting axial budding to varying degrees depending on the specific mutations. We identified one CDK-dependent phosphorylation site in Bni4 and have preliminarily identified interactions between Bni4 and the unconventional myosins Myo3/5 that are disrupted under conditions when Bni4 is phosphorylated. Together, these studies are elucidating specific roles for cytoplasmic mitotic CDK activity in connecting the cell division cycle to bud morphology and cell wall assembly.

83B. SUN Family Proteins Sun4p, Uth1p and Sim1p are efficiently secreted out of the *Saccharomyces cerevisiae* cells and regulated differently during development of yeast cultures. ***Evgeny Kuznetsov***[1], *Helena Kucerova*[2], *Zdena Palkova*[1], *Libuse Vachova*[2]. 1) Department of Genetics and Microbiology, Charles University in Prague, Czech Republic; 2) Institute of Microbiology of the ASCR.
SUN family includes 4 SUN genes (SIM1, UTH1, NCA3 and SUN4) coding for proteins with homology in their C-terminal amino acid domain (about 258 amino acid long). Although the functions of these proteins are mostly unknown, they have been linked to various cellular functions and processes such as cell wall/mitochondrial biogenesis, mitophagy, cell septation and oxidative stress resistance. Uth1p and Sun4p have dual cell wall/mitochondrial localization. We have shown recently (Kuznetsov et al., 2013, PLOS ONE) that Uth1p, Sim1p and Sun4p are efficiently secreted out of yeast cells occurring in particular growth phases and that the production of these proteins is dependent on the level of oxygen. Uth1p, Sun4p, Sim1p and Nca3p are synthesized during the growth phase of both liquid cell cultures and colonies growing on respiratory complete media. Cellular and extracellular variant of Sun4p have different level of modification (possibly glycosylation). Drop assay showed that SUN-protein-deficient yeast strains differ in properties of their cell walls. The strains individually deleted in each of the SUN genes have different sensitivity to yeast cytostatic compounds (Calcofluor

white, Congo red, SDS and boric acid) and these sensitivities differ when cells grow on different carbon sources. In addition, we show that deletion of any of the SUN genes increases yeast cell resistance to zymolyase treatment. In summary, our new data suggest that SUN-family proteins are regulated differently during particular phases of yeast culture development and under different environmental conditions, apparently being involved in remodeling of the cell wall and its resistance to extracellular compounds. In addition, the finding that cell sensitivity to boric acid, i.e. to a fungistatic compound often used in the medical treatment, is dependent on the presence (and level) of Uth1p, makes this protein an interesting target for studies of boric acids action. Supported by GAUK 903313.

84C. The Npr2 complex regulates a metabolic switch that controls TORC1 dependent proliferation during amino acid limited growth. ***Sunil Laxman***, *Benjamin Sutter, Lei Shi, Benjamin Tu.* Biochemistry, UT Southwestern Medical Center, Dallas, TX.
Under specific nutrient limited conditions where wild-type cells undergo autophagy, yeast cells lacking the Npr2/Npr3/Iml1 protein complex can bypass autophagy and continue to proliferate rapidly. The underlying metabolic and mechanistic basis of this hyper-proliferation in these cells remains unknown. We combined quantitative LC/MS/MS based metabolomic, genetic and biochemical approaches to dissect metabolic and mechanistic differences between prototrophic wild-type and Npr2 deficient yeast cells under these conditions. We found that yeast cells lacking the Npr2 complex have a metabolic state that dramatically contrasts with the metabolism of wild-type cells, reflecting their ability to rapidly proliferate. Through this metabolic switch, npr2 cells upregulate glutamine and S-adenosylmethionine metabolism, which enables cells to synthesize multiple metabolites required to sustain this rapid proliferation. These metabolic and proliferative changes in npr2cells occurred through the increased activity of the major eukaryotic cell growth regulator, the TORC1, through the activity of the Rag complex and Sch9 kinase. Our data thus reveal the metabolic transformation during rapid proliferation in npr2 cells as well as the mechanistic basis of how the Npr2 complex controls cell proliferation. This study highlights the role of glutamine metabolism, and shows how changes in nitrogen and sulfur metabolism need to be integrated during unchecked cell proliferation.

85A. Role for alkaline ceramidase and its products sphingoid bases in the oxidative stress response. *Jae Kyo Yi, Ruijuan Xu, EunMi Jeong,* ***Cungui Mao****.* The Department of Medicine and Cancer Center, Stony Brook University, Stony Brook, NY.
Reactive oxygen species (ROS), such as $O_2\cdot$, H_2O_2, and ·OH, are produced in the mitochondria of most all organisms, if not all, as a byproduct of normal oxidative phosphorylation. Accumulated ROS can potently cause loss of mitochondrial membrane potential and mitochondrial fragmentation, resulting in significant mitochondrial dysfunction including decreased oxygen consumption, decreased ATP production, and increased ROS production. In addition to a direct physical damage to this organelle, ROS, as signaling molecules, can activate various signaling pathways that may also cause the mitochondrial dysfunction indirectly.Because the mitochondrial dysfunction leads to unregulated apoptosis and premature cellular senescence, thereby various diseases, it is imperative to understand how ROS indirectly compromise mitochondrial structure and function. Emerging evidence suggests that ROS can alter the homeostasis of sphingolipids, some of which, act as signaling molecules to induce apoptosis by altering the mitochondrial structure and function. Oxidative stress has been shown to enhance the formation of sphingosine (SPH), a sphingolipid, in rat retina neurons and blocking the formation of SPH inhibits the apoptosis of neurons. Accumulated SPH in turn has been shown to induce ROS in cells and tissues. These results suggest that ROS and SPH form a positive feedback loop. However, it is unclear how this positive feedback loop is formed in cells. Because SPH is essentially generated from the hydrolysis of ceramides through the action of ceramidases and SPH has been shown to compromise the mitochondrial function, we hypothesize that ROS induces SPH generation by activating a ceramidase and that SPH in turn induces ROS generation by altering the mitochondrial structure and function. This hypothesis was tested using a yeast Saccharomyces cerevisiae system. We demonstrated that treatment with H_2O_2 increased the expression of YPC1, a yeast alkaline ceramidase that is responsible for the production of phytosphingosine (PHS), the yeast SPH. Knocking out YPC1 markedly attenuated yeast growth inhibition in response to H_2O_2. In contrast, overexpression of YPC1 induced mitochondrial fragmentation, ATP drop, ROS production, and growth inhibition by accumulating PHS in cells. These results suggest that YPC1 plays an important role in the oxidative stress response by disrupting the mitochondrial structure and function in a sphingoid base-dependent manner. This study provides a novel insight into the mechanism by which ROS induces mitochondrial dysfunction and may facilitate our understanding of the pathogenesis of diseases associated with oxidative stress.

86B. The unfolded protein response has a protective role in yeast models of classic galactosemia. *Evandro A. De-Souza, Felipe S.A. Pimentel, Caio M. Machado, Larissa S. Martins, Wagner S. da-Silva, Mónica Montero-Lomelí,* ***Claudio A. Masuda****.* Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
Classic galactosemia is a human autosomal recessive disorder caused by mutations in the *GALT* gene (*GAL7* in yeast), which encodes the enzyme galactose-1-phosphate uridyltransferase. Using two yeast models of galactosemia - lithium-

treated cells and the *gal7* mutant - we show that the unfolded protein response pathway is activated by galactose. The synthesis of galactose-1-phosphate is essential to trigger the unfolded protein response because the deletion of the galactokinase-encoding gene *GAL1* completely abolishes unfolded protein response activation and galactose toxicity. Impairment of the unfolded protein response in both yeast models makes cells even more sensitive to galactose, unmasking a cytotoxic effect. These results indicate that endoplasmic reticulum stress is induced under galactosemic conditions and underscores the importance of the unfolded protein response pathway in the cellular adaptation of these models of classic galactosemia.

87C. Measurement of thiamine vitamers and ethanol/CO2 in C. glabrata. ***Erin M. Neal***, *Christine L. Kerwin-Iosue, Dennis Wykoff.* Biology Department, Villanova University, Villanova, PA.
Thiamine biosynthetic pathway in *Saccharomyces cerevisiae* and *Candida glabrata* has important differences both in terms of biosynthetic genes and in the regulation of those structural genes. We hypothesize that changes in thiamine metabolism between the two species alter the relative flux of carbon through fermentation and oxidative phosphorylation pathways. Measuring the end products of respiration (CO2) and fermentation (ethanol and CO2) in addition to thiamine cellular concentrations using HPLC & EtOH/CO2 sensors has allowed us to correlate metabolites and test our hypothesis. For HPLC measurement of thiamine derivatives, we used a C16 amide column with an isocratic flow of methanol/phosphate buffer, and detected 0.125-1.0 mM concentrations of thiamine. We utilized sensors from Vernier for EtOH and CO2. This study aims to evaluate the differences in thiamine biosynthesis of *C. glabrata* compared to *S. cerevisiae* in high and no thiamine growth conditions, while also deleting genes known to be necessary for the conversion of thiamine to TPP (metabolically active cofactor made from thiamine). We confirmed that we can measure thiamine, thiamine monophosphate (TMP) and thiamine pyrophosphate (TPP) with a calibration curve of thiamine, and singly and doubly phosphorylated forms of thiamine by HPLC (with an r20.95). We are testing the following expectations. In high thiamine conditions, we expect *C. glabrata* to show high levels of thiamine and TPP but little to no levels of TMP because in biosynthesis thiamine gets converted to TPP without a TMP intermediate. Moreover, we expect *C. glabrata* to generate less EtOH than *S. cerevisiae* and to generate more CO2. In no thiamine conditions, we expect *C. glabrata* will have lower levels of thiamine relative to *S. cerevisiae* because of a lack of a biosynthetic arm of the pathway. As expected, we saw a decrease in TMP of both *C. glabrata* and *S. cerevisiae* in high thiamine conditions as well as a decrease in all thiamine derivatives for *C. glabrata* in no thiamine conditions. We are able to observe differences in EtOH and CO2 end products, but they do not appear to support our hypothesis as *C. glabrata* appears to evolve large amounts of EtOH. We are exploring alternate growth conditions to determine whether thiamine metabolism impacts the flux of carbon through changes in fermentation vs. respiration.

88A. Identification of regulators of riboneogenesis by high-throughput metabolomic and expression screens. ***Yoomi Oh***[1,2], *Adam Rosebrock*[1], *Amy Caudy*[1,2]. 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.
The newly discovered riboneogenesis pathway provides Saccharomyces cerevisiae, among other fungi, with an alternate route for the synthesis of ribose-5-phosphate without affecting cellular redox balance. Components of this pathway are co-regulated during the yeast metabolic cycle, yet specific regulators of core riboneogenic enzymes have not been identified. We have conducted genetic screens for regulators of the riboneogenesis pathway using complementary readouts of cellular metabolite levels and protein abundance to probe how cells regulate this pathway. We used high throughput liquid chromatography - tandem mass spectrometry to determine the levels of key metabolic inputs and products of the riboneogenesis pathway in each overexpression strain from a high copy plasmid collection. In parallel, we are using high-throughput quantitative flow cytometry to screen for regulators of Shb17, the committed step in the riboneogenesis pathway. We are querying the effect of nonessential gene deletions, temperature sensitive alleles of essential genes, and individual gene overexpression on a fluorescent reporter of Shb17 expression. Using these complementary approaches, we will identify the genetic regulators of riboneogenesis to better understand control of this newly identified pathway.

89B. The fourth function of the cell wall. ***Hiroki Okada***, *Shinsuke Ohnuki, Yoshikazu Ohya.* Integrated Biosciences, University of Tokyo, Kashiwa, Chiba, Japan.
The cell wall of the budding yeast *Saccharomyces cerevisiae* is a rigid structure serving as a barrier to extracellular environment. It is widely believed that there are three major functions of the cell wall during yeast life cycle. The wall gives a cell its osmotic integrity, defines cell shape, and provides a scaffold to present adhesive glycoproteins to other yeast cells. In this report, we present the evidence showing fourth function of the cell wall. To thoroughly understand the functions of the cell wall, we used image analysis software CalMorph to quantitatively analyze cellular phenotypes from 501 morphological viewpoints after treatment with cell-wall-affecting drugs Echinocandin B (EB), Tunicamycin (TN) and Nikkomycin Z (NZ), which block cell wall biosynthetic pathways. Extraction of morphological traits with notable drug effects, we found greater variance in some traits of drug treated cells (EB, TM or NZ, P 0.05, Jonckheere-Terpstra test). To investigate overall phenotypic variations, we scored to compare the distribution of phenotypic variance with and without

the drugs, and found that more than half of all morphological traits showed broad distribution after drug treatment. General profile of phenotypic variance was unique to each drug, but we found some common-traits (e.g. mother-to-daughter cell size ratio) to all drugs were increased after drug treatments. Based on these analyses, we concluded that yeast cell wall has a role in preventing phenotypic variation, especially in cell size control. Yeast deletion mutants with highly variable phenotypes were previously described (Levy and Siegal, 2008, PLoS Biol). In their list, mutants of many genes responsible for cell wall construction exhibited higher variability among all non-essential deletion mutants (e.g. *anp1* mutant defective in subunit of the -1,6 mannosyltransferase complex showed the 2nd highest in the list). We explored whether variance of the drug induced common-traits was became higher in cell wall defective mutant. We found such mutants in all major cell wall components biosynthetic pathways, such as mannoprotein defective mutant (e.g. *anp1*), 1,3--glucan synthase catalytic subunit mutant (e.g. *fks1-ts*), 1,6--glucan synthesis mutant (e.g. kre6), and chitin synthesis mutant (e.g. *chs1*). Although the molecular mechanism remains unclear, the cell wall structure may provide robustness to the cell by protecting the intracellular functional network from environmental conditions.

90C. Roles of four putative Cyk3-binding proteins in coordination of cleavage-furrow ingression and abscission during cytokinesis. ***Masayuki Onishi***, *Meng Wang, John Pringle.* Dept Gen, Stanford Univ, Stanford, CA.
Cytokinesis in *S. cerevisiae* is carried out in two steps: in the first step, the chitinous primary septum (PS) is formed at the division site, promoting cleavage-furrow ingression and actomyosin-ring constriction; the secondary septum (SS) then fills the small gap left in the center of the PS, thus completing cytokinesis (abscission). Cyk3, Hof1, and Inn1 form a complex that is involved in PS formation; this complex also interacts with the IQGAP protein Iqg1 and the type-II myosin Myo1. In addition, Cyk3 has a role in coordinating sequential formation of PS and SS by regulating Rho1 activity. We found that disruption of Cyk3-Inn1 binding by mutations in either the PXXP motif of Inn1 or the SH3 domain of Cyk3 had little effect on the ability of either Inn1 or Cyk3 to function. However, the mutant Cyk3 was no longer able to serve as a dosage suppressor of *iqg1* and *inn1* mutations, suggesting that the Cyk3 SH3 domain has binding partners other than Inn1 that are involved in its function. A previous global survey had identified four possible binding partners, Aim44, Nba1, Nis1, and Iml1, and we examined the possible roles of these proteins in cytokinesis. Consistent with such a role, Aim44, Nba1, and Nis1 each localized to the division site as a ring during cytokinesis. Although Iml1 appeared to localize primarily to the vacuolar membrane as previously reported, a weak localization as a ring to the division site during cytokinesis was also observed. These rings did not constrict together with Myo1, indicating that these proteins are not components of the actomyosin ring. Deletions of the four genes, either alone or in combination, affected SS formation and caused mild cell-clumping phenotypes. All four of the genes showed significant genetic interactions with *CYK3* and either *iqg1* or *inn1*. These results suggest that interactions between the Cyk3 SH3 domain and these four proteins have important roles in fine-tuning the timing of PS and SS formation.

91A. Contribution of metabolic adaptation and chronological aging to cell differentiation within yeast colonies. ***Zdena Palkova***[1], *Libuse Vachova*[2], *Michal Cap*[1], *Marcela Hejlova*[2]. 1) Department of Genetics and Microbiology, Charles University in Prague, 128 44 Prague 2, Czech Republic, zdenap@natur.cuni.cz; 2) Institute of Microbiology of the ASCR, v.v.i., 142 20 Prague 4, Czech Republic.
We have shown recently that yeast colonies are capable of differentiation to cell subpopulations differing in their properties and localization within the structure. Different cell types have been identified in smooth versus structured biofilm colonies [1, 2], which indicates that differentiation is differently regulated in the particular colony types. In aging smooth giant colonies formed by *Saccharomyces cerevisiae* laboratory strains, two major cell subpopulations have been identified [1]. Vital, long-living U cells localized in upper colony regions reprogram their metabolism and acquire various properties similar to tumor cells of mammals, while lower L cells are gradually dying and possibly provide nutrients needed for U cell survival. To determine contribution of active metabolic adaptation and of chronological aging to colony differentiation process, we analyzed U and L cell formation and properties in relatively young microcolonies performing the same developmental cycle as giant colonies, but in an accelerated manner [3]. We show that metabolic reprogramming important for longevity of upper cells in either giant colonies or microcolonies is a regulated process independent of chronological aging of these cells, but dependent on specific signals (e.g., NH_3) produced by the colony cell population. Knocking out of specific genes, as well as changes in properties of lower cells, negatively affect fitness of upper cells. The revitalization of upper cells that are derived from stressed cell ancestors includes re-activation of some pro-growth mechanisms including the TORC1 pathway. On the other hand, viability and stress-related properties of lower cells seem to be related to chronological aging and also to the time interval that these cells spent in vicinity of upper cell. The work was supported by GACR 13-08605S. *References*: [1] Cap M, Stepanek L, Harant K, Vachova L, Palkova Z (2012) Mol Cell 46:436-48; [2] Vachova L, Stovicek V, Hlavacek O, Chernyavskiy O, Stepanek L, Kubinova L, Palkova Z (2011) J Cell Biol 194: 679-87; [3] Vachova L, Hatakova L, Cap M, Pokorna M, Palkova Z (2013) Oxid Med Cell Longev 102485. doi: 10.1155/2013/102485.

92B. Roles of *FMS1* (orf19.4589) and *CBP1* (orf19.7323) genes in the *de novo* beta-alanine synthesis pathway from polyamines in *Candida albicans*. ***Ruvini U Pathirana***[1]*, Dhammika H. M. L. P. Navarathna*[2]*, Kenneth W. Nickerson*[1]. 1) School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE; 2) Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
Saccharomyces cerevisiae is capable of *de novo* pantothenic acid biosynthesis (metabolic precursor to coenzyme A), involving a unique pathway of beta-alanine synthesis from polyamines. The key enzyme in this pathway is a polyamine oxidase encoded by the Fenpropimorph Multicopy Suppressor gene 1, *FMS1* (YMR020W). The activity of some amine oxidases is inhibited by very minor quantities of farnesol and its analogs. Farnesol is the quorum sensing molecule in the human pathogenic yeast *Candida albicans* that inhibits yeast to filamentous morphological transition, which is an important virulence factor. Therefore, we investigated the role of the *FMS1* homolog in *C.albicans*; it could be an important regulatory mechanism of morphogenesis, and more speculatively a target for controlling this prevalent human pathogen. We have found that, in the presence of beta alanine, *C.albicans* accelerates germ tube formation in the N-Acetyl glucosamine containing differentiation medium. This finding suggests that beta alanine is synergistic for filamentation of this pathogenic yeast. In order to further investigate the role of beta alanine in *C.albicans* morphogenesis, we constructed a *FMS1*(orf19.4589) gene deletion strain of in *C.albicans* SC5314 background along with the complementation strain to restore the phenotype using the SAT1 flipper strategy. We also investigated some phenotypic characteristics such as morphology, drug sensitivity, stress responses and growth rates. For *fms1* deletion strains, the absolute requirement of beta-alanine or pantothenic acid was not observed under normal growth conditions in minimal media, in contrast to its homolog YMR020W in *S.cerevisiae*. This difference suggests that FMS1 in *C.albicans* is not the rate limiting step in *de novo* beta alanine synthesis as it is for *S.cerevisiae* and it could act together with a redundant gene *CBP1* (orf19.7323), that encodes a corticosteroid binding protein Cbp1p, which shares 35% identity with Fms1p of *S.cerevisiae*. These findings suggest that *C.albicans* exploits a redundant regulation in the beta-alanine biosynthesis pathway when compared to *S.cerevisiae* pathway, which might provide be an additional advantage for its pathogenic life style.

93C. Revisiting the myth of trehalose in the heat shock response: The Tps1 protein, and not trehalose, protected yeast cells from losing viability at high temperature. ***M. Petitjean****, MA. Teste, JM. François, JL. Parrou.* Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, UMR-CNRS5504 & UMR-INRA 792 & INSA & Université de Toulouse, Toulouse, France.
In *Saccharomyces cerevisiae*, trehalose is synthesized by a protein complex bearing a trehalose-6-phosphate synthase encoded by *TPS1* and a trehalose 6-P phosphatase encoded by *TPS2*. A mutant lacking *TPS1* is known to exhibit extreme heat shock sensitivity, a trait associated with the lack of trehalose (1, 2). We wished to revise this hypothesis by constructing an inactive catalytic variant of Tps1p, which allowed us to uncouple the function of the protein itself from the role of its two products, T6P and trehalose. We validated the total loss of the catalytic activity of this Tps1* variant and showed that the protein was correctly expressed in a *tps1* mutant prior to carry out heat shock experiments at 42C. As expected, *tps1* mutant lost 60 % of viability after 2 hr at 42C, whereas the wild type cell viability remained around 80%. Remarkably, the loss of viability of a *tps1* mutant was fully prevented by the expression of the inactive catalytic variant Tps1*. To confirm the requirement of the Tps1 protein independently of trehalose synthesis, we complemented the *tps1* null mutant by the *E.coli* homologous of *TPS1* that allowed production of trehalose during heat shock. In spite of the synthesis of the disaccharide, the *tps1* strain expressing OtsA exhibited a dramatic drop of viability upon heat shock at 42 C, exactly like the *tps1* null mutant. Moreover, accumulation of trehalose by cultivating the *tps1* null mutant on a trehalose medium did not protect this mutant against heat stress. Altogether, these results disclosed an unexpected and critical role of Tps1 protein in survival to heat shock at 42C, which is independent of its catalytic activity. Moreover, this function is specific to the yeast Tps1 protein since it cannot be brought about by the E coli homolog of Tps1 encoded by OtsA.
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94A. Revisiting the role of *TPS1* encoding trehalose 6-P synthase in the regulation of yeast glycolysis. ***M. Petitjean****, A. Vax, MA. Teste, JL. Parrou, JM. François.* Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés ,UMR-CNRS5504, UMR-INRA 792,INSA, Toulouse, France.
In spite of remarkable molecular and biochemical researches on glucose metabolism, all attempts to control and enhance the glycolytic carbon flux in the yeast *Saccharomyces cerevisiae* by rational modifications of the pathway has failed. This failure points out to our still incomplete understanding of the the regulation of this central metabolic pathway in this highly relevant biotechnology yeast. Bearing this in mind, *TPS1* encoding the trehalose-6-P synthase is of particular relevance in yeast glycolytic flux. Indeed, loss of *TPS1* function causes growth defect on rapid fermentable sugars, apparently due to hyper-accumulation of sugars phosphates and depletion of ATP. Two models have been proposed to account for this

phenotype, namely (i) a restriction of the glucose influx due to the inhibition of hexokinase by trehalose 6-P (T6P), the product of the reaction catalyzed by Tps1 and (ii) the Pi-regeneration associated with trehalose cycling. However, none of these models can explain other phenotypes or metabolic defects of this mutant, such as sporulation defect, hyper glycogen accumulation, respiration chain malfunctioning, etc. Using a catalytically inactive variant of Tps1 termed Tps1*, we show that the hyper-accumulation of sugars phosphate and ATP depletion upon glucose addition to respiratory growing *tps1* mutant were restored to wild type levels upon expression of the Tps1* variant. In spite of this metabolical rescue, *tps1* tps1* strain did not fully recoverfermentation capacity and its growth resumption on glucose was severely delayed. On the other hand, the *tps1* tps1* strain expressing OtsA protein, the E. coli homologous of Tps1, was able to recover almost normal fermentation capacity and to rapidly grow on fermentable sugars, whereas a *tps1* mutant transformed with OtsA alone did not recover normal metabolite levels upon glucose addition and stopped growth on this sugar after more than 10 hours. Taken together, these data shed new light on the mechanism by which Tps1 controls yeast glycolysis, proposing a new model in which the TPS system is composed of two distinct modules: module 1- the protein Tps1 is indispensable for growth on fermentable sugars and could even have additional function on its own (see abstract on heat shock response), whereas module 2 -T6P- is needed for the rapid switch between respiration and fermentation, and as such may contribute to the so-called Crabtree effect.

95B. Specific histone residues mediate *RTS1* rescue of *gcn5* growth under stress. ***Emily L. Petty***[1,2], *Shannon M. Tomlinson*[1], *Anne Lafon*[3], *Bryce Mendelsohn*[4], *Kristofor Webb*[1], *Eric J. Bennett*[1], *Lorraine Pillus*[1,2]. 1) Division of Biological Sciences, UCSD, La Jolla, CA; 2) Moores Cancer Center Institute, UCSD, La Jolla, CA; 3) Institut Curie, Paris; 4) Department of Pediatrics, Division of Medical Genetics, UCSF, San Francisco, CA.
Post-translational modification (PTM) of proteins relays information between and within cells. Signaling cascades send information into the cell to promote conrolled timing of cellular events. PTMs of histones, the major constituents of the DNA-protein complex known as chromatin, serve similar signaling functions in the genome to direct chromatin-templated events in addition to regulating access to DNA sequence information. Although the functions of PTMs are diverse, one common characteristic is their dynamic nature. Understanding the enzymes responsible for adding and removing PTMs to histones and other targets is an essential step for understanding the regulation and function of specific modifications. We have discovered a novel interaction between two highly conserved factors involved in PTM, the histone acetyltransferase (HAT) Gcn5 and the PP2A complex. One of two genes encoding PP2A regulatory subunits in budding yeast, *RTS1*, is a robust high-copy suppressor of *gcn5* temperature sensitivity. We have taken a combined genetic and biochemical approach to identify specific histone residues that undergo phosphorylation *in vivo* and are required for *RTS1* rescue. Specifically, we screened through the SHIMA core histone mutant library for residues required for *RTS1* rescue and analyzed extracted histones by Mass Spectrometry to identify phosphopeptides. We have also used a directed genetic screen to identify candidates for the relevant protein kinase in this interaction. Our current hypothesis is that dynamic histone acetylation and phosphorylation is required for normal progression through the cell cycle. This work may shed light on the relatively unexplored territories of histone serine, threonine and tyrosine phosphorylation and specific phosphatases that regulate dynamic histone phosphorylation.

96C. Characterization of double budding in wild-type *Saccharomyces cerevisiae*. ***Angela L. Piotrowski***, *Robert M. Seiser*. Roosevelt University, Schaumburg, IL.
The principle of singularity in cell polarization holds that *S. cerevisiae* undergoing normal asexual reproduction will produce a single bud and give rise to a single daughter per cell cycle. Mutation or altered expression of certain genes involved with cell polarization, such as those encoding the Rho-like GTPase Cdc42, the scaffold protein Bem1p or the nucleotide exchange factors Gea1/Gea2p and Cdc24p, can cause dysregulation of bud site selection. In a small percentage of affected cells, a loss of singularity is manifested as two buds emerging from the same mother cell within one cell cycle. This double-budding phenomenon can nonetheless give rise to two viable daughter cells. The aim of this study was to identify, validate and characterize the extent to which wild-type cells are capable of demonstrating double-budding morphology. Using fluorescence microscopy, quantitative image analysis and flow cytometry, we show that commonly used laboratory wild-type strains, as well as several viable deletion mutants derived from these strains, can produce two viable buds simultaneously under certain growth conditions. Our results indicate a defined period of increased double-budded cell emergence in wild-type cells, which occurs in early logarithmic phase under optimal growth conditions in rich media. In contrast, cells overexpressing Bem1p display double-budding at a low but consistent frequency throughout vegetative growth. Wild-type cells were also found to follow normal bud site selection patterns in both proximal and distal polarizations. The molecular processes underlying double-budding in wild-type cells are still unclear, though the phenomenon may be influenced by cell density and nutrient availability in relatively young cultures.

97A. Ubiquitylation may promote the Glc7 activity opposing Ipl1 kinase during mitosis. ***R. Ravindran***[1], *P. Polk*[2], *L. C. Robinson*[1], *K. Tatchell*[1]. 1) Biochemistry and Molecular Biology, LSUHSC, Shreveport, LA; 2) Research Core Facility, LSUHSC, Shreveport, LA.

Prior to anaphase, dynamic kinetochore-microtubule interactions are regulated by reversible phosphorylation mediated by the kinase/phosphatase pair, Aurora B/PP1 (Ipl1/Glc7 in *S. cerevisiae*). Loss of Ipl1 activity results in failure to form bipolar kinetochore-microtubule attachments and loss of the spindle assembly checkpoint (SAC), causing lethality due to massive chromosome loss. Loss of the opposing Glc7 activity results in activation of and failure to terminate the SAC. Many suppressors of conditional *IPL1* alleles alter Glc7 or its regulators. We determined the complete genome sequence of an uncharacterized *ipl1-2* suppressor mutant from a large mutant screen. Using this sequence, we identified the suppressor mutation as a missense mutation in *UBA1* (*uba1-W928R*), encoding the single essential E1 ubiquitin-activating enzyme. Trp928 is conserved in all E1 proteins and is located in a flexible linker between the main body of the E1 enzyme and the docking domain for E2 ubiquitin-conjugating enzymes. Given the conservation and location of W928, *uba1-W928R* is likely to result in reduced levels of substrate ubiquitylation. Consistent with this idea, the *uba1-W928R* mutant has a pleiotropic phenotype, including slow growth, thermosensitivity, and sensitivity to UV radiation. We propose that a component(s) of the Aurora B/PP1 regulatory pathway is under-ubiquitylated in the *uba1-W928R* mutant. From immunoblot analysis of whole cell extracts, Ipl1 protein levels are 1.4 fold higher in *uba1-W928R* mutant cells than in wild-type cells, consistent with the possibility that the mutation may act by stabilizing the Ipl-2 protein. However, genetic evidence indicates that this increase cannot fully explain the suppression. Glc7 protein levels are not altered in *uba1-W928R* mutant cells, but several lines of evidence suggest that Glc7 may be regulated by ubiquitylation. First, the subcellular localization of Glc7-mCitrine is altered in *uba1-W928R* mutant cells, with relatively more Glc7 in the cytoplasm than in the nucleus. Second, the *uba1-W928R* mutation is lethal in combination with several mutant alleles of *GLC7* that were identified as suppressors of *ipl1-2*. Furthermore, one of the PP1 ubiquitylation sites identified in yeast and human proteomic screens (yeast K112, human K113) was mutated in one of our previously identified suppressors of *ipl1-2* (*glc7-K112E*). These and other observations lead us to conclude that ubiquitylation somehow activates Glc7 in its role opposing Ipl1 at the kinetochore.

98B. The efflux pump MlcE from the *Penicillium solitum* compactin biosynthetic gene cluster increases *Saccharomyces cerevisiae* resistance to natural statins. ***Ana Rems***, *Rasmus John Normand Frandsen.* DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark.
The use of statins as cholesterol-lowering drugs is based on their ability to inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), the key enzyme in the mevalonate pathway, which is responsible for the production of ergosterol in fungi and cholesterol in human. Industrial scale production of natural statins (i.e. compactin and lovastatin) and their semi-synthetic derivatives (i.e. pravastatin and simvastatin) is based on fermentation of statin-producing filamentous fungi, such as *Aspergillus terreus* and *Penicillium solitum*, however, the unique physiology and morphology make these natural producers difficult to culture in bioreactors. The production limitations associated with the use of natural producers can be overcome by heterologous expression of the biosynthetic pathway in *Saccharomyces cerevisiae* (1), however, it is crucial to establish a nondestructive resistance mechanism in yeast, which would overcome the undesirable effects of statins. One possible mechanism is an active export of statins, a mechanism that does not just provide the resistance but can also significantly ease the purification of the produced compounds. In order to establish export of statins from yeast we integrated a putative efflux pump-encoding gene *mlcE* from the *P. solitum* compactin biosynthetic gene cluster into *S. cerevisiae* genome. The resulting strain was tested for susceptibility to statins by growing the strain on media containing statins. The constructed strain showed an increased resistance to both natural statins (compactin and lovastatin), and also to a semi-synthetic statin simvastatin, when compared to the wild type strain. Expression of a mRFP-tagged MlcE show that MlcE is localized in the yeast plasma membrane. In conclusion we provide evidence indicating that MlcE is a transmembrane efflux pump, capable of exporting natural and semi-natural statins from yeast, and overexpression of MlcE in a statin-producing yeast could therefore greatly improve the commercial production of natural and semi-natural statins.
Reference: (1) Xu W. et al., (2013), LovG: The Thioesterase Required for Dihydromonacolin, Angew. Chem. Int. Ed. 2013, 52, 6472 -6475.

99C. Determining the adaptive landscape of the SUL1 promoter. ***Matthew S. Rich***[1], *Celia Payen*[1], *Maitreya J. Dunham*[1], *Stanley Fields*[1,2,3]. 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, University of Washington, Seattle, WA.
Mutations can affect any of several processes to make organisms more fit. For example, gene expression can be modulated through copy-number variation or promoter mutations, whereas gene function can be modulated through only coding mutations. To evaluate how these different mutational processes create fitter individuals, we use an in vivo yeast selection that typically results in gene amplification. When evolved under sulfur-limited conditions, yeast amplify the sulfate transporter SUL1 and the resultant increased protein expression confers a distinct growth advantage. This amplification is highly reproducible, occurring in nearly all sulfate-limited evolution experiments. No advantageous single nucleotide variation has been found at the SUL1 locus, and it is unknown whether such variation can be as adaptive as amplification. To address this issue, we are analyzing the fitness of a library of comprehensively mutagenized SUL1 promoters. Through

truncation analysis, we defined the SUL1 promoter to be at most 493 bases long, though a promoter ~100 base pairs shorter is sufficient if yeast have an additional copy of the SUL1 gene. We created a barcoded library of approximately 137,000 variants of this promoter, which we will assay for fitness in a pooled competition. By assaying many of the possible sequence variants of the SUL1 promoter, we hope to identify alternative mutational pathways to gene amplification that yield similar phenotypic outcomes.

100A. Physiological role of nitric oxide as a signaling molecule in the regulation of sporulation in Saccharomyces cerevisiae. ***Kyohei Saiki***, *Akira Nishimura, Iwao Ohtsu, Daisuke Watanabe, Hiroshi Takagi.* NAIST, Nara, Japan.
Nitric oxide (NO) is a ubiquitous signaling molecule involved in the regulation of a large number of cellular functions. In the unicellular eukaryote yeast, NO may be involved in stress response pathways, but its role is poorly understood due to the lack of mammalian NO synthase (NOS) and soluble guanylate cyclase (sGC) orthologues in the genome. We recently revealed a novel antioxidative mechanism mediated by NO in Saccharomyces cerevisiae Σ1278b background strain. Our results indicated that increased conversion of L-proline into L-arginine led to NO production in response to elevated temperature, thereby inducing intracellular reactive oxygen species (ROS) generation. We also found that the flavoprotein Tah18, which was previously reported to transfer electrons to the Fe-S cluster protein Dre2, was involved in NO synthesis, as a yeast NOS-like protein. Interestingly, Tah18-dependent NO biosynthesis confers high-temperature stress tolerance on yeast cells (Nishimura et al., BBRC, 2013). Here, to uncover the downstream pathways of NO in S. cerevisiae, we focused on the cGMP-mediated signaling pathway, which is activated by sGC. When yeast cells were treated with 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), which is a commonly used sGC inhibitor, cell growth was severely inhibited. We also found that the pharmacological inhibition of NO biosynthesis and the cGMP pathway significantly reduced the spore formation rate of diploid cells. These results raise the possibility that NO production in S. cerevisiae might be required for sporulation during meiosis through the cGMP-dependent signaling pathway.

101B. New insights into glucose sensing by S. cerevisiae. ***Kobi Simpson-Lavy***, *Mark Johnston.* Biochemistry and Molecular Genetics, CU-Denver School of Medicine, Aurora, CO, 80045.
S. cerevisiae responds rapidly to glucose availability by inducing signaling pathways that lead to expression and activation of hexose transporters and fermentation, and to repression of respiration. One of these pathways employs transporter-like glucose sensors (Snf3/Rgt2) that signal via Casein Kinase (Yck1/2) and the Rtg1 transcriptional repressor to regulate hexose transporter expression. We identified several novel components of this SRR (Snf3/Rgt2/Rgt1) signaling pathway downstream of the glucose sensors: The Snf1 (AMP-activated) protein kinase inhibits signaling in the SRR pathway by phosphorylating Yck. The Snf1-interacting protein Sip5 recruits Snf1 to the sensors to inhibit signaling, and the Vhs1 protein kinase relieves this inhibition in response to high levels of glucose by phosphorylating Sip5. These results lead us to a new model of the SRR signaling pathway that shows how the cell integrates signals from three different glucose sensing pathways.

102C. A minimal glycolytic pathway in *Saccharomyces cerevisiae*. ***D. Solis-Escalante***[1], *N. Barrajón Simancas*[1], *N.G. Kuijpers*[1], *J.T Pronk*[1,2], *JM. Daran*[1,2], *P. Daran-Lapujade*[1]. 1) Department of Biotechnology, Delft University of Technology, Delft, The Netherlands; 2) Platform Green Synthetic Biology, Delft, The Netherlands.
Despite several decades of research, our understanding of glycolysis is far from complete. In *Saccharomyces cerevisiae* the 12 biochemical reactions for the conversion of glucose to ethanol are catalysed by 26 enzymes. The individual contribution to glycolysis of those iso-enzymes remains unclear and the glycolytic regulatory mechanisms are still elusive. In an attempt to reduce the complexity of the glycolytic pathway, a yeast strain carrying a minimal glycolytic pathway (called MG) was constructed. A minimal set of 13 genes required for a functional glycolysis was selected, leading, with the exception of phosphofructokinase that requires both *PFK1* and *PFK2*, to a single gene per glycolytic step. The MG strain was constructed by the sequential deletion of 13 genes using newly developed molecular tools for selectable marker excision and recycling[1] avoiding LoxP/Cre induced chromosome translocations. Remarkably, under controlled conditions and nutrient excess, measurement of growth performance, extra- and intracellular metabolites profiles and glycolytic enzyme activities, revealed that the deletion of 13 glycolytic paralogs did not affect *S. cerevisiae*. This lack of phenotypic response was also observed at the transcriptome-wide level. Our data demonstrate that the genetic redundancy in *S. cerevisiae* glycolysis is not required for normal growth and response under a wide range of cultivation conditions. While it has been described that simple, targeted genetic manipulations can have a strong impact at the whole genome level[2], the 13 deletions and 11 marker recycling rounds only marginally altered the genome of MG strain. As little as 13 mutations were observed, none of them within glycolytic genes or regulators. This study provides a powerful eukaryotic platform strain in which glycolytic enzymes can be easily swapped for any glycolytic homologue. This platform should prove very valuable for complementation analysis, drug target design or kinetic modelling.
[1]Solis-Escalante *et al*. 2013. *FEMS Yeast Res*. 13(1):126-39. [2]Teng *et al*. 2013. *Mol Cell*. 52(4):485-94.

103A. Improvement of ethanol stress tolerance in an industrial fuel-ethanol *Saccharomyces cerevisiae* strain. *A. Bucker, J. C. do Espirito-Santo, G. Muller, M. G. Dario,* ***B. U. Stambuk***. Dept Biochemistry, Univ Federal de Santa Catarina, Florianopolis, Santa Catarina, Brazil.
The impending depletion of fossil resources, and fossil fuel combustion as the primary cause of global warming, have sharply increased global demand for fuel ethanol. The capacity to produce high levels of ethanol is a very rare characteristic in nature, and it is most prominent in the yeast *Saccharomyces cerevisiae*. High ethanol tolerance is crucial for the use of yeasts in the fermentation industries, since it strongly influences the rate and completion of fermentation. Ethanol tolerance in yeast has been extensively studied and revealed that properties like membrane lipid composition, chaperones, vacuolar and peroxisome function, and trehalose content are important determinants of ethanol tolerance. Genome-wide transcriptomics and screening of deletion mutants have revealed many genes required for tolerance to ethanol in laboratory yeast strains. Aiming to improve ethanol tolerance of an industrial fuel ethanol yeast [1,2], we have analyzed the ethanol tolerance and fermentation performance of a laboratory and the industrial strain with overexpression (by genomic engineering) of the *TRP1* [3], the *MSN2*, or a truncated form of this gene, lacking the initial 50 amino acids [4]. Our results show that while the laboratory strain is sensible to 10% ethanol, the industrial yeast strain could tolerate 14% ethanol, and overexpression of the *TRP1*, *MSN2* (or its truncated form) genes improved growth under higher ethanol concentrations. Fermentations of high sugar concentrations in the absence or presence of stressful ethanol concentrations revealed that the laboratory strain overexpressing the genes had a significant better fermentation performance, but the same was not observed with the industrial strain. Thus, our results indicate that different genetic elements regulate ethanol tolerance and ethanol production in industrial fuel ethanol yeasts. Financial support: CNPq, CAPES and FINEP. [1] Stambuk et al., Genome Res. 19: 2271 (2009). [2] Babrzadeh et al., Mol. Genet. Genomics 287: 485 (2012). [3] Hirasawa et al., J. Biotechnol. 131: 34 (2007). [4] Hong et al., J. Biotechnol. 149: 52 (2010).

104B. Feral strains forming biofilm colonies are derived from domesticated strains under stress conditions. ***Libuse Vachova***[1]*, Zdena Palkova*[2]*, Vratislav Stovicek*[2]*, Marketa Begany*[1]. 1) Institute of Microbiology of the ASCR, v.v.i., 142 20 Prague 4, Czech Republic, vachova@biomed.cas.cz; 2) Department of Genetics and Microbiology, Charles University in Prague, 128 44 Prague 2, Czech Republic.
Wild strains of *Sacharomyces cerevisiae* preferentially form structured biofilm colonies possessing a number of protective strategies [1]. Among others these strains are also able to reprogram themselves in nutrient surplus by a process called domestication and subsequently start to form smooth colonies. This domestication is accompanied by switching off of most of the protective processes [2]. Here we show that the domestication is a reversible process [3]. Under conditions of long-lasting starvation of domesticated strains, the feral clones start to appear with low frequencies. Feral strains resemble the original wild strains as regards their colony morphology and architecture, including formation of extracellular matrix, production of Flo11p adhesin and ability to absorb high amount of water; i.e., the features that are switched off during the domestication. However, cell morphology of feral strains differs from wild strains, being more similar to domesticated variants. In addition, the frequency of domestication is higher in feral strain when compared with the wild strains. This work was supported by GACR 13-08605S and the Ministry of Education, Youth and Sports of the Czech Republic project CZ.1.07/2.3.00/20.0055 from the European Regional Development Fund in the Czech Republic. *References*: [1] Vachova et. al (2011) J Cell Biol 194: 679-87; [2] Stovicek et al (2010) Fungal Genet Biol 47: 1012-22; [3] Stovicek et al (2014) BMC Genomics. 15:136. doi: 10.1186/1471-2164-15-136.

105C. Identification of yeast Greatwall kinase Rim15p as a novel negative regulator for alcoholic fermentation. ***Daisuke Watanabe***[1,2]*, Yan Zhou*[2]*, Aiko Hirata*[3]*, Yoshikazu Ohya*[3]*, Takeshi Akao*[2]*, Hitoshi Shimoi*[2]*, Hiroshi Takagi*[1]. 1) NAIST, Nara, Japan; 2) NRIB, Hiroshima, Japan; 3) University of Tokyo, Kashiwa, Japan.
Alcoholic fermentation by the budding yeast *Saccharomyces cerevisiae* is one of the most familiar and industrially-important microbiological processes. To elucidate the regulatory mechanism *in vivo* for alcoholic fermentation, we performed whole genome DNA sequencing and transcriptomic analyses of a representative sake-brewing yeast strain Kyokai no. 7, which exhibits the high fermentation rate and the high ethanol production yield. As we previously reported (Watanabe *et al.*, *Appl. Environ. Microbiol.*, 2012), the *rim15*^{5055insA} loss-of-function mutation is predominantly responsible for the fermentation properties of sake strains, identifying Greatwall-like protein kinase Rim15p as a novel negative regulator of alcoholic fermentation. So far, we have revealed that deletion of *RIM15* or its orthologous gene improved the fermentation rates of *S. cerevisiae* bioethanol strain PE-2 and wine strain EC1118, *S. pastorianus* lager strain Weihenstephan 34/70, and also *Scizosaccharomyces pombe*. To understand how loss of the Rim15p functions enhances the fermentation rate in Saccharomyces cerevisiae, we investigated intracellular carbon metabolism during fermentation using the CE-TOFMS technique. The most outstanding feature of the *rim15* cells was decreased synthesis of UDP-glucose, which is catalyzed by UDP-glucose pyrophosphorylase Ugp1p. Rim15p activates stress-responsive transcription factors Msn2/4p and Hsf1p, both of which upregulate the expression of *UGP1* mRNA and the glucose anabolic pathway to accumulate storage (trehalose and glycogen) and structural (1,3--glucan) carbohydrates. We found that defective glucose anabolism caused by downregulation of *UGP1* or deletion of the phosphoglucomutase genes *PGM1/2* significantly

increased the fermentation rate. Consistently, sake strain Kyokai no. 7 with the *rim15*^{5055insA} mutation and the outstanding fermentation properties exhibited impaired synthesis of trehalose, glycogen, and 1,3--glucan. These results demonstrate that Rim15p is a key regulator in metabolic switching from glycolysis toward glucose anabolism. It is also noteworthy that the sake strains share a common loss-of-function T1086A mutation in the *GLG2* gene, encoding glycogenin glucosyltransferase. This supports the idea that the glucose anabolic genes might be actually disused in the sake strains. Altogether, our results provide novel insights into the Rim15p-mediated stress responses as major impediments of effective alcoholic fermentation, which are widely applicable to the development and improvement of advanced yeast strains in the bakery, brewing, and biofuel industries.

106A. Re-inventing central carbon metabolism in *Saccharomyces cerevisiae* for high-volume production of farnesene. ***Kati Wu***, *Kristy Hawkins, Yoseph Tsegaye, Adam Meadows, Lauren Pickens, Anna Tai, Tina Mahatdejkul-Meadows, Eugene Antipov, Madhukar Dasika, Lan Xu, Lily Chao, Savita Ganesan, Jefferson Lai, Patrick Westfall, Timothy Gardner, Annie Tsong.* Amyris Inc., Emeryville, CA.
-Farnesene is a commercially valuable molecule whose derivatives can be used in a wide variety of applications ranging from fuels to novel performance chemicals. At Amyris, we have used the native glycolytic pathway of *Saccharomyces cerevisiae* and a heterologous farnesene synthase to produce farnesene at industrial scale from plant-derived sugars. Generating farnesene using the native pathway, however, results in an imbalance of pathway metabolites and cofactors, translating to high energetic costs to the cell and overall higher production costs. Here, we design and construct an alternative metabolic pathway in *S. cerevisiae* which reduces the cost of converting glucose to farnesyl pyrophosphate (FPP), the precursor to farnesene and all other sesquiterpenes. By dividing glucose dissimilation between parallel heterologous routes, and by altering the cofactor requirements of FPP biosynthesis, we dramatically improve internal pathway balance, reducing the demand to maintain redox homeostasis and energy charge using side reactions. The result is a 20% improvement in the theoretical yield of farnesene from glucose (g/g), and a 170% improvement in the theoretical yield from oxygen (mol/mol). We introduced our synthetic metabolic network into a strain of S. cerevisiae previously engineered for commercial-scale production of farnesene. In this strain background, we observed an 80% increase in the molar yield of farnesene from oxygen, which translates directly to an 80% increase in the volume of farnesene that can be produced per unit time from fixed fermentation capacity under industrial oxygen-limited conditions. This improvement in productivity enables microbial production of farnesene at commercial scale with significantly reduced cost.

107B. An “oncometabolite” in yeast: 2-hydroxyglutarate accumulates in response to altered mitochondrial and central carbon metabolism. ***Olga Zaslaver***[1,2], *Adam Rosebrock*[1], *Amy Caudy*[1,2]. 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.
Recent studies have identified gain-of-function mutants of IDH1 (isocitrate dehydrogenase) in multiple human tumours. Cells harbouring this mutant enzyme accumulate D-2-hydroxyglutarate (2-HG) resulting from endogenous -ketoglutarate. The oncometabolite 2-HG has been implicated in promoting tumour cell growth and has been demonstrated to allosterically regulate a range of enzymatic activities including prolyl hydroxylases and histone demethylases. 2-HG is present in healthy cells in small quantities; despite a flurry of theories of how accumulation of D-2-HG affects cancer progression, little research has been done to explore the biochemical origin and fate of endogenous 2-HG.
During the course of an untargeted mass spectrometry screen for novel enzymes in yeast, I identified multiple loss-of-function mutants that accumulated an unknown metabolite that we subsequently confirmed to be 2-HG. Using a combination of genetics, chemical perturbation, and high resolution liquid chromatography mass spectrometry (LC-MS), I examined the origin and role of 2-HG in carbon flux and metabolism as a whole. We have used a combination of in vitro enzyme assays, yeast genetics, and stable-isotope kinetic flux to determine which enzymes are responsible for production of 2-HG and have quantified the contribution of each enzyme to cellular 2-HG levels. We find that 2-HG concentrations increase when cells grow on anaplerotic nitrogen sources that direct additional carbon into the citric acid cycle, but are reduced in conditions of obligate respiratory growth. Future research will focus on elucidating the relationship of this molecule to cellular metabolism, downstream physiological state, and organism fitness.

108C. Linker scanning mutagenesis of microtubule nucleating components Spc97 and Spc98. ***Kimberly Fong***[1], *Jerry Tien*[1], *Celia Payen*[2], *Alex Zelter*[1], *Beth Graczyk*[1], *Maitreya Dunham*[2], *Trisha Davis*[1]. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA.
The mitotic spindle is a large structure responsible for the regulation and organization of chromosome segregation. Microtubules play several key roles during mitosismediating chromosomal movements, stabilizing spindle structure, and positioning the spindle within the cell. The -tubulin small complex is a highly conserved complex, essential for microtubule nucleation, comprised of two -tubulin molecules and one copy each of Spc97 and Spc98. Despite our advances in understanding microtubule function, dynamics and nucleation, purified -tubulin complexes fail to efficiently recapitulate nucleation *in vitro*, suggesting that additional regulatory processes activate the nucleating complex. Based on

EM structure, we hypothesize that Spc98 undergoes a conformational change that enables the -tubulin small complex to serve as a template for microtubule growth. We have performed a saturating linker-scanning mutagenesis to define the regions of Spc97 and Spc98 required for the small complex to function *in vivo* and promote the formation of a microtubule. *In vitro* transposition reactions generated a library of random 15 base pair insertions in each gene, with approximately 90% coverage. An extensive library was collected and transformed into yeast to screen for viability and conditional lethality using a plasmid shuffle. Mutants that abolish function identify the essential regions of the protein and mutants that are temperature sensitive can be studied after shifts to restrictive temperatures to examine nucleation activity. Over 50,000 yeast colonies were screened and 4% of the colonies contained lethal mutations and 0.02% contained temperature-sensitive mutations. Sites of lethal insertions were identified and mapped by high-throughput sequencing. Representative lethal mutations have been studied to determine the effects on protein expression, localization, and small complex formation. Temperature sensitive mutants were inspected by fluorescence microscopy and *in vitro* nucleation assays to reveal several nucleation hyperactive and deficient mutants. When combined with high-throughput sequencing, linker-scanning mutagenesis was a powerful approach to identify essential regions of the -tubulin complex and provide novel temperature-sensitive mutants with specific defects in microtubule nucleation.

109A. Regulation of the kinetochore localization and activity of the protein kinase Mps1 during mitosis. ***Lori Koch***[1,2], *Sue Biggins*[2]. 1) Molecular and Cellular Biology, University of Washington, Seattle, WA; 2) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.
Faithful chromosome segregation depends upon the ability of cells to make correct kinetochore-microtubule attachments. When there are defects in these attachments, the cell activates a mechanism known as the spindle checkpoint that halts progression into anaphase until errors are corrected. The conserved protein kinase Mps1 phosphorylates many proteins of the kinetochore and has been shown to play an essential role in the spindle checkpoint and error-correction. The mechanism by which Mps1 localizes to and maintains itself at kinetochores during mitosis has remained elusive. Making use of a technique our lab has developed to purify intact budding yeast kinetochore particles, we are using genetic and biochemical techniques to dissect the requirements for Mps1 occupancy on the kinetochore. Furthermore, we are testing how defects in Mps1 localization may lead to defects in the spindle checkpoint and error-correction pathways. Finally, we aim to study how Mps1 checkpoint activity or kinetochore localization is altered upon microtubule attachment in biochemical and biophysical reconstitution experiments.

110B. Kinetochores require oligomerization of the Dam1 complex to maintain microtubule attachments against tension during biorientation. ***Neil Umbreit***[1], *Matthew Miller*[2], *Jérôme Cattin-Ortolá*[1], *Jerry Tien*[1], *Charles Asbury*[3], *Trisha Davis*[1]. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Department of Physiology and Biophysics, University of Washington, WA.
Kinetochores assemble on centromeric DNA and present multi-copy arrays of components that attach directly to the dynamic ends of spindle microtubules. These microtubule-binding kinetochore components coordinate autonomously at the microtubule interface through oligomerization, but how their oligomerization contributes to kinetochore function has remained unclear. Using a combination of biophysical assays and live-cell imaging, we found that oligomerization of the Dam1 kinetochore complex is required for its ability to form microtubule attachments that are robust against tension *in vitro* and *in vivo*. An oligomerization-deficient Dam1 complex that retains wild-type microtubule binding activity was specifically defective in coupling to disassembling microtubule ends under mechanical load *in vitro*. In cells, kinetochore-microtubule attachments mediated by the oligomerization-deficient Dam1 complex failed under the tensile forces experienced during bipolar alignment of sister chromatids. We propose that oligomerization is an essential and conserved feature of kinetochore components that is required for accurate chromosome segregation during mitosis.

111C. Quality Control of Higher-order Septin Assembly: Mapping the Septin Proteostasis Network in vivo. ***Andrew Weems***, *Michael McMurray.* Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO.
Septins are a family of GTP-binding cytoskeletal proteins that assemble into filamentous rings in many eukaryotic cell types. At the bud neck in S. cerevisiae these rings act as scaffolds to recruit factors necessary for cell division, and as diffusion barriers that compartmentalize the plasma membrane. Mutant septins incapable of binding GTP are prevented from incorporating into rings when wild-type molecules of the same protein are available, but the details of this quality control system remain unknown. Our data point to a primary role for GTP in de novo septin folding, and suggest that cytosolic molecular chaperones maintain quality control of septin assembly. To better understand the interplay between nascent septin proteins and cytosolic chaperones, we are employing a variety of in vivo techniques centered around the generation of proteomic and localization data using bimolecular fluorescence complementation (BiFC). Additionally, we have designed a novel system to monitor step-wise the progress of newly-expressed proteins through chaperone networks in living yeast. Using these techniques, we seek to not only learn which chaperones facilitate septin folding and potentially

assist in the sequestration of misfolded septins, but to answer fundamental questions about the temporal and spatial dynamics of cytosolic proteostasis.

112A. Crosslinking analysis identifies hundreds of distance constraints in protein complexes. ***Alex Zelter***[1], *Michael Hoopman*[2], *Richard Johnson*[3], *Michael MacCoss*[3], *Robert Moritz*[2], *Trisha Davis*[1]. 1) Department of Biochemistry University of Washington, Seattle, WA; 2) Institute for Systems Biology, Seattle, WA; 3) Department of Genome Sciences, University of Washington, Seattle, WA.
High-resolution structural information on proteins and their interaction networks is required to understand the mechanisms underlying cellular processes. Chemical cross-linking in combination with mass spectrometry analysis (XL-MS) has great potential for rapidly generating high-resolution structural information and accurately defining large protein interaction networks. Until now XL-MS methods have been inaccessible to most mass spectrometry labs. Here we describe Kojak, an algorithm that: (i) includes simple to use, effective and fully automated software that can just be run on the MS data acquired, (ii) provides accurate and automatic differentiation between true and false positive identifications in a statistically valid manner and (ii) can be used with samples treated with commercially available crosslinking reagents without isotope labeling. We show that this method can generate hundreds of accurately identified distance constraints and provide important structural information about protein complexes not amenable to other approaches. We will present the data for the Ndc80 complex and the Dam1 complex.

113B. Regulation of the Ndc80 complex during meiosis in budding yeast. ***Jingxun Chen***[1], *Angelika Amon*[2], *Elcin Unal*[1]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.
The kinetochore is the large protein complex that mediates chromosome segregation. Regulations on its assembly and function ensure faithful genome propagation. In budding yeast, premature expression of the B-type cyclin Clb3 during early prophase I disrupts the meiosis I-specific chromosome segregation pattern. The defects associated with *CLB3* misexpression can be suppressed by transiently inactivating the kinetochore, suggesting a potential interplay between the kinetochore and Clb3. To understand this interplay, we characterized the effect of *CLB3* misexpression on the four subunits of the Ndc80 complex, which generates the microtubule-binding interface of the kinetochore. We found that among the four subunits, Ndc80 protein levels declined during prophase I in wild-type cells, but were stabilized upon *CLB3* misexpression. The decline of Ndc80 required Aurora B and proteasome activity. Expression of *NDC80* and *CLB3*, but not *CLB3* alone, during late prophase I caused chromosome segregation defects. These results are consistent with the notion that *NDC80* is down-regulated in prophase I to prevent premature microtubule-kinetochore interactions, thereby helping the establishment of a meiosis I-specific chromosome segregation pattern.

114C. To What Extent Does Homologous Chromosome Pairing Depend on Recombination-Independent Chromosome Interactions? ***S. Cheng***, *A. MacQueen.* Wesleyan University, Middletown, CT.
During meiosis, homologous chromosomes undergo a dynamic series of interactions that ultimately lead to their segregation apart from one another at the first meiotic division. In order to properly segregate, chromosomes must first recognize, and then establish a stable paired association with their homologous partners. The process by which homologs recognize one another is still uncertain, however, one hypothesis is that chromosomes actively search for their partner through transient associations. In budding yeast, it is clear that homologous recombination is central to the homolog pairing process. However, a set of recombination-independent chromosome interactions also occurs during early meiotic prophase I, and we want to understand the extent to which meiotic homolog pairing relies on these early, recombination-independent chromosomal interactions. During a short period in early meiosis, telomeres (chromosome ends), gather to a small region of the nuclear membrane resulting in bouquet formation. Ndj1 is a telomere- associated protein that is required for bouquet formation in budding yeast. In ndj1 mutants homolog pairing is achieved after a delay, suggesting that the efficiency of homolog pairing may rely on bouquet formation. Furthermore, during early meiosis centromeres undergo pairwise interactions, independent of homology, via a process called centromere coupling. Centromere coupling is mediated by Zip1, a component of a meiosis-specific chromosomal structure called the synaptonemal complex. Like ndj1, zip1 mutants show robust homolog alignment at later stages of meiosis, but whether homolog pairing is delayed in zip1 mutants has not been established. In order to assess the combined contribution of bouquet formation and centromere coupling to the recombination-dependent homolog pairing process, we are analyzing homolog pairing at a centromeric and a chromosomal arm region in meiotic cells that lack bouquet formation (ndj1), centromere coupling (zip1), or both processes (ndj1 zip1). Our analysis monitors chromosome pairing via lacO and tetO arrays that are positioned at the centromere and an arm region of chromosome IV (in conjunction with tagged LacI and TetR proteins). Our pairing analysis is carried out via a time course in order to determine the efficiency of homolog pairing during meiotic progression in these mutant backgrounds. We are furthermore assessing chromosome pairing in cells that are also missing NDT80 and thus cannot progress beyond late meiotic prophase. Pairing analysis at late time points in ndt80 mutants allows us to assess the maximum pairing levels that ndj1, zip1 and ndj1 zip1 strains are capable of achieving, regardless of pairing efficiency.

115A. Regulation of nuclear shape in response to mating pheromone. *Alison Walters, Emma Dauster,* ***Orna Cohen-Fix***. NIDDK/NIH, LCMB, Bethesda, MD.
The nuclear envelope (NE) of budding yeast is dynamic: during interphase the nucleus is round, but as cells enter mitosis the NE expands, allowing nuclear elongation and chromosome segregation to take place. Changes in nuclear morphology also occur in response to mating pheromone, where the nucleus elongates. In both budding and fission yeast there is a constant ratio between nuclear volume and cell volume, but whether this affects nuclear shape isn't clear. The mechanisms that regulate NE expansion are not understood. We identified temperature sensitive mutants that, at the non-permissive temperature, failed to elongate their nucleus in response to alpha factor mating pheromone. These mutants were in genes involved in fatty acid synthesis (*fas1*, *fas2* and *acc1*), suggesting that nuclear elongation during mating requires membrane synthesis. Indeed, when comparing wild type cells arrested in G1 with or without alpha factor, the elongated nuclei of cells exposed to alpha factor increased in surface area but not in volume. We propose that during the mating response yeast actively increase their nuclear surface area, but in order not to increase nuclear volume nuclei become elongated rather than expand isometrically. When wild type cells were released from the alpha factor induced arrest, nuclei became round as cells progressed through the cell cycle. In doing so, they increased in volume but not in surface area. These observations suggest that yeast alter their nuclear shape by regulating the surface area of the nucleus. What might be the role of nuclear elongation during mating? When *fas2* mutant cells of opposite mating types were mixed, cells were able to form normal-looking shmoos, suggesting that cell shape changes, unlike nuclear shape changes, can occur with the existing fatty acid pools. Despite the round nuclear shape, the efficiency of nuclear fusion in *fas2* cells was similar to that of wild type cells, suggesting that nuclear elongation is not necessary for nuclear fusion. Zygotes of *fas2* mutants, however, arrested prior to the first mitosis (a phenotype not seen with cycling *fas2* cells), suggesting the increase in surface area during mating is needed for nuclear division after nuclear fusion.

116B. Developmentally-programmed mega-autophagy drives mother cell demise during yeast gametogenesis. ***Michael Eastwood****, Marc Meneghini.* Moleular Genetics, University of Toronto, Toronto, Ontario, Canada.
Recently, we reported our discovery of programmed nuclear destruction (PND) during S. cerevisiae gametogenesis, or sporulation. PND involves the destruction aborted meiotic nuclei through an apoptotic-like DNA fragmentation pathway coupled with the autophagic degradation of nuclear protein. While exploring autophagic mechanisms contributing to PND, we found that the rupture of the lysosome-like vacuole is a programmed event which is intrinsic to sporulation. Upon the completion of meiosis, the vacuoles of the mother cell are not segregated to into daughter spores. During spore formation the vacuole retained by the mother becomes catastrophically engorged, to the point of lysis, driving the demise of the mother cell as it transitions into the sac-like structure present in the mature ascus. Vacuolar lysis, also referred to as mega-autophagy, represents a common mechanism of programmed cell death in plants and is analogous to animal cell death pathways which engage lysosomal membrane permeabilization. We now hypothesize that the mega-autophagy-driven destruction of the meiotic mother cell represents a de facto programmed cell death fundamental to sporulation. To gain insight into the regulation of mega-autophagy during sporulation, we examined vacuolar integrity in mutants arrested at various stages of meiotic development. We find that upon the commitment to the meiotic nuclear divisions, a step controlled by the master transcriptional regulator Ndt80, the mother cells vacuoles coalesce into a single enlarged vesicle which expands during spore formation and eventually lyses. Cells which lack Ndt80 remain arrested at the pachytene stage and possess multiple intact vacuoles. We hypothesize that the Ndt80-dependent developmental program which underlies mid-sporulation promotes the abandonment of the typical balance of vacuolar fission and fusion in favor of enhanced vacuolar fusion. Additionally, we find that mega-autophagy requires the sporulation-specific MAP kinase Smk1. While Smk1 is required for the construction of the outer spore wall, we find that other spore wall mutants execute mega-autophagy normally, suggesting that Smk1 promotes mother cell demise independently of its role in spore wall development. Our ongoing studies continue to test the hypothesis that mega-autophagy-mediated mother cell death is controlled by an alteration of vacuolar dynamics coupled with a MAP kinase-dependent signal originating from the spore.

117C. Role of CWI-pathway and Rlm1 transcription factor in colony sporulation patterns. *Sarah Piccirillo, Rita Morales, Melissa G. White, Keston Smith,* ***Saul M. Honigberg***. Cell Biol/Biophysics, School Biol Sciences, Univ Missouri-Kansas City, Kansas City, MO.
A screen for mutants that were defective in sporulation in colonies but not in cultures identified several mutants in genes of the cell-wall integrity (CWI) pathway (MPK1/SLT2, BCK1, and SMI1/KNR4). We found that the CWI pathway was induced as colonies mature, and was required for sporulation to initiate in colonies. In wild type colonies, sporulation occurs in a highly organized pattern, with two sharply defined layers of sporulating cells separated by a layer of lightly staining somewhat enlarged cells. We suggest that the lightly staining cell layer contains non-sporulating feeder cells. Feeder cells retained viability during the period that sporulation occurs in colonies, but were sensitive to low osmolarity, indicating that these cells may have increased permeability. The increased osmo-sensitivity in colonies depended on Rlm1, a transcription factor that defines one branch of the CWI pathway. Rlm1 was also required for the pattern of sporulation within colonies. Finally, Rlm1 acted through a cell non-autonomous mechanism to regulate initiation of sporulation in

colonies. These results suggest that as colonies develop, the CWI pathway is required for efficient formation of a feeder cell layer, and that the permeable cells in this layer provide an overlying cell layer with nutrients and/or other signals required for efficient sporulation.

118A. Mating and mating-type determination in the yeast *Kluyveromyces marxianus*. ***H. Hoshida****, S. Murashige, S. Tokuda, R. Akada*. Dept Appl Mol Biosci, Yamaguchi Univ, Ube, Yamaguchi, Japan.
Kluyveromyces marxianus is a thermotolerant yeast suitable for bioethanol production at high temperatures. For industrial application, breeding of *K. marxianus* through mating and sporulation is a promising approach but mating of *K. marxianus* has not been reported. We investigated mating and mating type determination in *K. marxianus*. Two auxotrophic mutant strains with different nutritional requirements, which were obtained from the same wild type strain, were crossed on several kinds of agar media, incubated, and replica-plated on minimal medium (MM) plates to select diploid cells. After a few days incubation, colonies were formed on MM plates, indicating that *K. marxianus* derived from one parental strain can mate, thus is homothallic. The diploid cells produced spores and the progenies showed 2 by 2 separation of the auxotrophies. Mating efficiency was examined in liquid media containing various concentrations of yeast nitrogen base (YNB), ammonium sulfate, and glucose. The optimal condition was 0.17% YNB and 2% glucose. Unexpectedly, lower cell concentrations were necessary for efficient mating. To know the effect of pheromones and receptors, the genes encoding the and **a** pheromones and the receptors were disrupted and resulting disruptants were used for mating test. Mating of the pairs of *mf1* mutants, *ste2* mutants, and *ste3* mutants was failed, indicating that mating-factor receptors and -factor are indispensable for mating. However, *mf***a***1* mutant pair showed weak mating, suggesting that **a**-factor is dispensable. On the genome sequence of *K. marxianus*, three *MAT*-like loci were found but not the *HO* gene, indicating that *K. marxianus* has HO-independent switching mechanism. To observe mating types of the cells, both Km*MFA1* promoter-driven yEGFP gene and Km*MF1* promoter-driven yEmRFP gene were introduced. GFP and RFP fluorescence in a cell indicate a and type, respectively. In addition to the cells displaying either green or red fluorescence, a part of the cells showed both green and red fluorescence simultaneously. This result suggests that mating type of *K. marxianus* might be fluctuating. Possible advantages of the fluctuating mating-type determination in *K. marxianus* will be discussed.

119B. dHJ-C, a Tool for Genome-Wide Mapping of Homologous Recombination and Template Choice During Yeast Meiosis. ***Neil A. Humphryes****, Tovah Markowitz, Xiaoji Sun, Viji Subramanian, Andreas Hochwagen.* Biology, NYU, NYC, NY.
Meiosis is a feature of sexual reproduction, which generates haploid gametes from diploid cells. Following DNA replication, ~160 programmed DNA double-strand breaks (DSBs) are formed and repaired by homologous recombination (HR). During mitosis, HR almost always uses an identical sister chromatid as a repair template, however template bias alters during meiosis, and inter-homologue (IH) recombination is actively promoted. IH recombination is a source of genetic variation, as regions of DNA from each parent become reciprocally exchanged. To date, meiotic template choice has been observed at discrete loci, particularly the HIS4:LEU2 hotspot in budding yeast, which exhibits a 5:1 IH bias in WT meiosis. Several factors responsible for maintaining this balance have been identified using this single locus. Genome-wide studies have been successfully performed to determine meiotic DSB distribution and outcomes of meiotic recombination, but the recombination process between these two events remains elusive. It is likely that there is highly regulated spatial and temporal variation in recombination, as would be expected from the non-uniform distribution of meiotic DSBs. I am developing dHJ-C, a tool that maps genome-wide homologous recombination intermediates and determines template choice. dHJ-C is based on Hi-C, a genome-wide approach to observe the 3D conformation of chromatin. For dHJ-C, meiotic recombination intermediates, particularly double Holliday Junctions (dHJ), are stabilized and processed to allow high-throughput sequencing to quantify their abundance and genomic location. A hybrid diploid budding yeast strain whose parents exhibit 0.7% sequence divergence was employed to determine the parental origin of dHJ-proximal sequences. This hybrid has been used previously to map meiotic recombination outcomes between homologues, however dHJ-C will be able to identify both IH and IS recombination. I am currently optimizing dHJ-C and thus far I have successfully mapped recombination intermediates across the genome, which appears to correlate well with DSB distribution data. IH vs. IS bias has also been observed, showing weaker IH bias than observed previously at HIS4:LEU2. Intriguingly there appears to be variation in bias across the genome.

120C. Translational regulation determines gene insulation during yeast meiosis. ***Liang Jin****, Rolf Sternglanz, Aaron Neiman.* Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY.
In response to nutritional starvation, diploid yeast cells undergo meiosis and differentiate into haploid spores. Commitment is defined as the transition point during sporulation at which cell fate becomes independent of environmental signals. That is, committed cells will complete sporulation and produce spores even if they are transferred back to rich medium. Surprisingly, even in committed cells, transfer to rich medium causes extensive down-regulation of the transcripts of meiotic and sporulation genes. For only a small subset of these genes, termed insulated genes, the mRNA levels remain high even after re-introduction of nutrients. We demonstrate a strong correlation between insulation of genes after

reintroduction of nutrients and delayed translation during a normal sporulation. These two phenomena are genetically coupled as mutants that affect translational regulation also remove insulation. The 5' UTR of the *SPS4* gene is required both for insulation and for translational delay. We propose a model in which sequestration of certain transcripts both delays their translation and protects them from degradation upon re-introduction of nutrients. The significance of insulation for meiotic commitment is being investigated.

121A. Multiple MAPK cascades regulate the transcription of IME1, the master transcriptional activator of meiosis in Saccharomyces cerevisiae. ***Yona Kassir***[1], *Smadar Kahana-Edwin*[2], *Michal Stark*[1]. 1) Dept Biol, Technion Inst, Haifa, Israel; 2) Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel 52621.
Entry into meiosis in S. cerevisiae depends on the expression and activity of a master regulator, Ime1. The transcription of IME1 is regulated by all the meiotic signals through an atypical large and complex 5' region. In this report we focus on a distinct cis-acting regulatory element UASru, whose activity is regulated by multiple signals. A glucose signal inhibits UASru activity through the cAMP/protein kinase A pathway and the transcription factors (TF), Com2 and Sko1. A nitrogen source repressed UASru activity through the TF Sum1. Our results demonstrate that all the known MAPK cascades that operate in vegetative cultures directly affects UASru function: The Hog1 MAPK transmits a high osmolarity signal to UASru through the Sko1 TF; The Mpk1 MAPK transmits an elevated temperature signal through the TFs Swi4/Mpk1 and Swi4/Mlp1; Fus3 and Kss1 MAPKs transmit novel nutrient signal to UASru through the Ste12/Ste12 and Ste12/Tec1 TFs, respectively. This signal is specific to UASru and did not affect the mating and filamentation response elements that are regulated by these MAPK and TFs. Moreover, UASru did not respond to the mating and filamentation signals. Thus, the three optional developmental pathways available to yeast, namely, meiosis, filamentation and mating are regulated by the same MAPKs in a specific manner. A hypothesis aimed to explain how specificity is achieved will be discussed.

122B. Schizosaccharomyces japonicus provides the second example where chirality of DNA strands causes sister-sister cells developmental asymmetry. ***Amar J S Klar***. Gene Reg & Chromosome Biol Lab, NCI-Frederick Cancer Res Fac, Frederick, MD.
We championed DNA strand chirality as a unique mechanism of asymmetric cell division based on our fission yeast Schizosaccaharomyces pombe research. This has remained the only example perhaps because the existence of such a mechanism could not be experimentally tested in diploid organisms. As a consequence, the morphogen gradients model constitutes a major paradigm for explaining development in higher eukaryotes, but the model has remained non-established and controversial. We found that the DNA chirality mechanism indeed operates in evolutionarily very distant yeast, S. japonicus. The DNA strand based imprinting mechanism is remarkably conserved even though these organisms contain mat cassettes highly rearranged, one makes 4-spored and the other makes 8-spored asci, and protein orthologs exhibit only 50% amino acids identity between them. We will present numerous advantages that S. japonicus provides, as compared to the commonly used S. pombe and S. cerevisiae yeasts, as a model research organism. The commonly used yeast species exhibit a generation time of ~1 hr. 30 min and a genetic cross takes over a weeks time but S. japonicus grows with a generation time of 63 min and the genetic cross we accomplished in 2.5 days. These technical advantages of S. japonicus will be highlighted in the presentation and a start up kit of its strains will be made available to the conferees. We recognized that the mechanism of asymmetric cell division that gives rise to the phenomenon of mat1 switching could also explain vertebrate developmental differentiation. We had advanced a strand-specific imprinting and selective chromatid segregation model (SSIS) to achieve cellular differentiation by causing asymmetric cell division in diploid organisms, including mammals. Indeed we and others have provided evidence for selective chromatid segregation mechanism operating in mitoses of eukaryotes, the phenomenon we first invoked in our SSIS model. Thus lessons learned form studies with model yeast systems have been used to explain body development, such as visceral and brain laterality development of vertebrates and humans and to explain the psychoses etiology in humans.

123C. A mating pathway deficiency as a reproductive barrier between *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. ***Camille Meslin***[1], *Allyson O'Donnell*[2], *Nathan Clark*[1]. 1) Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA; 2) Cell Biology, University of Pittsburgh, PA.
A major mechanism by which speciation events are thought to occur is through barriers to gamete recognition thereby preventing the formation of species hybrids. *Saccharomyces cerevisiae* (*Scer*) and *S. paradoxus* (*Spar*) coexist in the same habitat and can produce inter-species hybrids, yet *Scer* shows clear preference towards its own species. *Spar*, however, does not demonstrate a clear preference between the two species. Yeast gametes produce pheromones directed at the opposite mating type so as to signal readiness for mating and *Scer* choose its mating partner with the stronger level of pheromone. In *Scer*, the binding of the pheromone to its receptor triggers a MAPK cascade that leads to the expression of specific genes, cell cycle arrest and eventually cell fusion and mating. In *Scer*, after pheromone exposure, MATa cells exhibit a typical morphology (shmoo) and Fus3p accumulates in the nucleus. To assess the efficacy of the mating signaling pathway in *Spar*, we quantified Fus3p accumulation for all the clades along *Spar* phylogeny and found a

heterogeneous response to the pheromone, even within the same clade. Some strains accumulate Fus3p in their nucleus and some do not. We also observed that the morphologies of certain *Spar* strains are atypical, namely some strains adopt more of a filamentous morphology than a canonical shmoo. We will now assess mating efficiency and the ability to arrest the cell cycle for each of the *Spar* strains to characterize the phenotype more deeply. To provide proper inter-specific contrasts we will also conduct the same experiments on wild isolates of *Scer*. Differences in mating kinetics is thought as a possible premating barrier between *Saccharomyces* species, *Scer* being faster to mate than *Spar*. In *Scer*, pheromone sensing is essential for cells to assess their potential mates. The apparent defect in *Spar* pheromone signaling pathway could explain that *Spar* is slower to mate and does not demonstrate a clear preference between its own gametes or those of *Scer*. We argue that *Spar* mating defect could be an effective prezygotic reproductive barrier. In addition, the *Spar* phenotype could be an exciting model to study the rewiring of signaling pathways.

124A. A developmental stage-specific Vps13 complex regulates phsophatidylinositol-4-phosphate pools. ***Jae-Sook Park***, *Aaron Neiman.* Biochem & Cell Biol, SUNY Stony Brook, Stony Brook, NY.
In vegetatively growing yeast, Vps13 localizes to endosomes where it is involved in the delivery of proteins to the vacuole. During sporulation, Vps13 relocalizes to the prospore membrane, where it is required for multiple aspects of prospore membrane morphogenesis: 1) Vps13 promotes membrane expansion via activation of the phospholipase D, Spo14; 2) *VPS13* is required for a late step in closure of the prospore membrane, the cytokinetic event that gives rise to spores; and 3) *VPS13* regulates a membrane bending activity that can generate intralumenal vesicles. We provide evidence that all of these effects are caused by reduced levels of phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate in the prospore membrane in the *vps13* mutant. Use of mass spectrometry to identify proteins that specifically co-immunoprecipitate with Vps13-GFP indentifies several Vps13 binding proteins including Spo71, a sporulation-specific PH domain protein. Deletion of *SPO71* causes phenotypes very similar to those seen in the *vps13* mutant. In a *spo71* mutant, Vps13 protein fails to localize to the prospore membrane and ectopic expression of *SPO71* in mitotic cells results in release of Vps13 from the endosome. These results suggest a model in which induction of *SPO71* during sporulation relocalizes Vps13 from the endosome to the prospore membrane where a complex of the two proteins influences the phosphatidylinositol metabolism. Finally, shRNA-mediated knockdown of the mammalian homolog *VPS13A* in PC12 cells results in reduction of phosphatidylinositol-4-phosphate levels at the plasma membrane, suggesting that regulation of this lipid is a conserved function of Vps13-family proteins.

125B. SPS1 and SPO77 act together to regulate prospore membrane closure during sporulation in S. cerevisiae. ***Scott Paulissen***, *Christian Slubowski, Linda Huang.* Biology, University of Massachusetts Boston, Boston, MA.
SPS1 encodes a STE20-kinase in the GCKIII subfamily known to be required for sporulation (Friesen, et al., 1994). We find that SPS1 plays a role in the morphology and development of the prospore membrane (PSM). The prospore membrane (PSM) is a double lipid bilayer that grows to surround the meiotic products formed during sporulation in S. cerevisiae. Previous work has demonstrated that AMA1, which encodes a meiosis-specific activator of the anaphase-promoting complex, and SSP1, which encodes a PI(4,5)P2 binding protein, are important for PSM closure (Diamond, et al., 2008; Maier, et al., 2007). Ssp1 and Don1 are found at the leading edge of the growing PSM and are part of the Leading Edge Protein (LEP) complex (Moreno-Borchart, et al., 2001). The removal of the LEP from PSM and the subsequent degradation of Ssp1 is believed to be necessary for the PSM to close (Maier, et al., 2007). We see that sps1 cells have a PSM closure defect and we find that this defect is exacerbated in the sps1 ama1 double mutant, suggesting that SPS1 and AMA1 act in parallel for proper PSM closure. Consistent with a role in PSM closure, we see that the LEP is not properly removed in sps1 mutants. We also identify SPO77 as a high copy suppressor of a hypomorphic sps1 allele, and find that SPO77 plays a similar role in PSM closure as SPS1. Epistasis analysis suggests that SPO77 acts with SPS1 during PSM development. Our data suggests a model where SPS1, acting with SPO77, regulates prospore membrane development by affecting prospore membrane closure through the regulation of the LEP. Diamond AE, Park J-S, Inoue I, Tachikawa H and Neiman AM. Mol Biol Cell (2009) 20: 134-145. Friesen H, Lunz R, Doyle S and Segall J. Genes Dev (1994) 8: 2162-2175. Maier P, Rathfelder N, Finkbeiner MG, Taxis C, Mazza M, LePanse S, Haguenauer-Tsapsis R, and Knop M. EMBO J (2007) 26: 1843-1852. Moreno-Borchart AC, Strasser K, Finkbeiner MG, Shevchenko A, Shevchenko A, and Knop M EMBO J (2001) 24: 6946-6957.

126C. Characterizing the function of Kar5p during inner nuclear membrane fusion during yeast mating. ***Jason V. Rogers***, *Mark D. Rose.* Department of Molecular Biology, Princeton University, Princeton, NJ.
During mating in the budding yeast *Saccharomyces cerevisiae*, two haploid cells fuse to form a diploid zygote. After cell fusion and nuclear congression, the two haploid nuclei fuse via two sequential, coordinated membrane fusion steps. SNAREs comprise the fusogen for outer membrane fusion, but the inner membrane fusogen remains unknown. The inner nuclear membrane fusogen would necessarily have at least three properties: be localized to the nuclear fusion site, be present in the inner membrane, and be essential for inner membrane fusion. Kar5p, identified in earlier genetic screens as being required for nuclear fusion, is a highly conserved transmembrane protein found in plants, fungi, and some animals

including zebrafish. Previous electron tomography studies suggested that Kar5p is partially required for both both outer and inner nuclear envelope fusion. Kar5p is oriented with a large domain in the lumen of the nuclear envelope and thus could potentially mediate inner nuclear membrane fusion. Taken together these observations make Kar5p a strong candidate for the inner nuclear membrane fusogen. To determine whether Kar5p is localized to both the outer and inner nuclear membranes at the site of nuclear fusion, we used a split-GFP assay pioneered by Sue Jaspersen. Kar5p localizes in a broad region over the outer nuclear envelope, but in a small patch next to the zone of nuclear fusion in the inner nuclear envelope. Kar5p is therefore at the correct location to be the inner membrane fusogen. Kar5p also recruits Prm3p, a protein required for outer nuclear membrane fusion, to the zone of nuclear fusion, explaining Kar5ps partial requirement for outer nuclear membrane fusion. We have created *kar5* alleles that successfully recruit Prm3p but still fail to rescue nuclear fusion, demonstrating that Kar5p must have extra functions beyond recruiting Prm3p. We are currently using electron microscopy to determine whether these *kar5* alleles specifically arrest at the inner nuclear membrane fusion step. During our mutation analysis we also identified a *kar5* allele in which Kar5p is expressed but fails to localize to the zone of nuclear fusion adjacent to the spindle pole body. In a complementary fashion, we demonstrated that the half-bridge protein Mps3p is required for Kar5ps localization. These insights have led to a new model of the nuclear fusion machinery, and suggest that Kar5p is the key fusogen for the inner nuclear membrane.

127A. Quantification of Meiotic Chromosome Missegregation Frequency in Natural Isolates of *Saccharomyces cerevisiae*. ***Amy Carol Sirr***, *Gareth Cromie, Aimée Dudley.* Pacific Northwest Diabetes Research Inst, Seattle, WA.
Meiotic chromosome missegregation is the underlying cause of a number of severe developmental syndromes, including Downs (trisomy 21) and Edwards (trisomy 18). Trisomies of chromosomes 21, 18, and 13 together account for approximately 1 in 556 live births and their frequency has been increasing over time. These frequencies stand in stark contrast to the high fidelity that has been measured in some model organisms, including *Saccharomyces cerevisiae*, where it is possible to isolate and analyze large numbers of meiotic progeny. One possible explanation is that nondisjunction frequencies in yeast have been determined in crosses between genetically similar parental strains and for a limited number of chromosomes. Our work with natural isolates of *S. cerevisiae* has uncovered some extremely high frequencies of chromosome missegregation, as high as 1 in 10 meiotic events in one cross between two genetically diverse, euploid strains. We are applying a genomic method called RAD-seq to the study of meiotic chromosome segregation. By sequencing the same 3% of each spores genome, RAD-seq provides a cost-effective means of determining ploidy and identifying crossover events. In the low fidelity segregation cross above, centromere-linked markers on the disomic chromosomes were overwhelmingly heterozygous, indicative of nondisjunction in meiosis I, a hypothesis further supported by the 2:2 spore viability observed in tetrads containing disomies. To extend characterization of meiotically occurring aneuploidy, we are applying high throughput methods, in which large numbers of tetrads tagged with a sporulation-specific reporter are isolated by flow cytometry followed by RAD-seq of individual spores, to a number of crosses with varying degrees of genetic diversity and starting levels of aneuploidy.

128B. Kel1p collaborates with Fus2p for yeast cell fusion. ***Jean Smith***, *Richard Stein, Mark Rose.* Molecular Biology, Princeton University, Princeton, NJ.
Cell fusion is ubiquitous in eukaryotic organisms. However, little is known about the molecular mechanisms controlling fusion. In yeast, cell fusion occurs when cells of opposite mating type form a diploid zygote. Fus2p is a pheromone-induced protein that regulates cell wall breakdown during fusion. Fus2p interacts with Rvs161p, and is transported to the shmoo tip, where it is anchored at the cortex. While mapping functional domains of Fus2p, we found that the last seven residues are required for localization, without affecting Rvs161p binding. Mutations in this region also cause severe mating defects. A screen for high copy suppressors of the C-terminal mutations identified KEL1. KEL1 encodes a kelch-domain protein, which was previously implicated in cell fusion, but its function in mating was not investigated further. We found that Kel1p overexpression allowed localization of the mutant Fus2p, suggesting that Kel1p might interact with Fus2p. To find the region of FUS2 required for localization suppression, we made successive truncations of the C-terminus. Analysis of the truncations suggested a model in which an auto-inhibitory looping mechanism inhibits an upstream localization region, which is also required for Kel1p suppression. This region contains a conserved motif, mutations in which block Kel1p suppression. Previous work showed that Fus2p is retained at the shmoo tip by both Fus1p- and actin-dependent pathways. Fus1p is a pheromone-induced membrane protein localized at the shmoo tip. We found that mutations at the C-terminus mainly affect the Fus1p-dependent pathway. Through deletion analysis, we found that Kel1p and its homologue, Kel2p, play a role in Fus2p localization, and we hypothesize that they act through the actin-dependent pathway. We have also found that Kel1p plays a secondary role in mating that is not through localization of Fus2p. Deletion of kel1 causes a cell fusion defect despite Fus2p being properly localized. Consistent with a second Fus2p-independent function, overexpression of Kel1p weakly suppresses a complete fus2. However, the much greater suppression of the C-terminal mutants argues that they are not being suppressed by a bypass mechanism. We conclude that Fus2p localization is dependent on both Kel1p and Fus1p, interacting through adjacent C-terminal domains and that Kel1p plays two different roles in the cell fusion pathway.

129C. *KAR4* has separable functions in mating and multiple steps of meiosis. ***Abigail J. Sporer****, Mark D. Rose.* Molecular Biology, Princeton University, Princeton, NJ.
Kar4p is required for both mating and meiosis. In mating, Kar4p works with Ste12p to activate the transcription of genes required for karyogamy, but its function in meiosis is unknown. To determine whether Kar4p has a distinct function in meiosis, we designed a genetic screen for mutant alleles that might differentially affect its function in each of these two pathways. We isolated equal numbers of mating-proficient, meiosis-defective and mating-defective, meiosis-proficient alleles, demonstrating that Kar4ps functions in mating and meiosis are independent. Characterization of the alleles revealed that all of the mating-defective alleles, but none of the meiosis-defective alleles, disrupted the Kar4p-Ste12p interaction. We conclude that all of Kar4ps mating functions are mediated by its role in transcriptional activation. Surprisingly, we find that Kar4p is required at least twice in meiosis. Cells without *KAR4* fail to initiate meiotic DNA replication, although the key early meiotic regulatory genes *IME1* and *IME2* are induced. However, overexpression of *IME1, IME2,* or *RIM4* can suppress this early defect, allowing cells to undergo DNA replication and recombination. A subset of the mutant kar4 alleles complete meiosis and form spores when *IME1* overexpression bypasses the first requirement for Kar4p. However, other alleles (including some defective for mating) remained blocked, apparently at the pachytene checkpoint, demonstrating that Kar4p is required for a second later function in meiosis. Mapping of the alleles on a threaded structure of Kar4p suggests that distinct regions of the protein are required for mating and each of the two meiotic functions. Kar4p also interacts with the meiotic RNA adenine methyl-transferase Ime4p. However, mutations that disrupt this interaction have no effect on meiosis, suggesting that the interaction is neither necessary nor sufficient for either of Kar4ps meiotic functions. We conclude that *KAR4* is a major regulator of at least two steps in the meiotic pathway, and that this role is independent from its function in mating.

130A. Unprogrammed presentation number.

131B. Investigating the roles of alternate and short ORFs in meiosis. ***Kelsey Van Dalfsen****, Gloria Brar.* Molecular and Cell Biology, UC-Berkeley, Berkeley, CA.
Proteins, the functional products of genome coding regions, have historically been identified by computational methods. Such annotation approaches have generally required a minimum length of approximately 100 amino acids, based on the assumption that this length is required for robust protein structure and function. Ribosome profiling is a new method that allows the first experimental annotation of open reading frames (ORFs) by measurement of ribosome positions on transcripts globally and in vivo. Ribosome profiling of yeast cells in meiosis revealed the translation of thousands of short ORFs, some of which represent alternate isoforms of well characterized proteins. All of these short meiotic ORFs are currently of unknown function and represent a large and promising new pool of factors with possible meiotic function. We are focusing on better understanding the range and significance of cellular functions for these short proteins, with the hope of gaining insight into the molecular basis for cellular remodeling during meiosis.

132C. Mitochondrial ribosome function/assembly: Regulation by accessory factors in *Saccharomyces cerevisiae*. ***Kaustuv Datta****, Jaswinder Kaur, Dharmendra Pandey.* Department of Genetics , University of Delhi South Campus, New Delhi, India.
Proper mitochondrial biogenesis and its optimal functioning are critical to numerous cellular processes including adaptation to nutrient availability and stress. Mitochondria require coordinated expression of nuclear and its own genome. Mitochondrial ribosomes are protein rich and rRNA deficient in comparison to their bacterial counterpart. Comparison of mitochondrial genome and predicted proteome indicates a direct correlation between increased ribosomal protein numbers and decreased rRNA size with the complexity of the eukaryote. Ribosome biogenesis requires numerous auxiliary factors that aid in maturation of precursor molecules into functional subunits. Our research is focused on understanding the regulation of mitochondrial ribosome function/biogenesis in *S. cerevisiae* by *YDR336w* and *YDR332w*. *YDR336w* is a putative GTPase that belongs to the YihA family of proteins with a clear ortholog in prokaryotes, lower eukaryotes and vertebrates only. Interestingly no orthologs are present in all other kingdoms of life including invertebrates and plants sequenced so far. Ydr336wp is predicted (60% probability) to be localized to the mitochondria. We have shown that *ydr336w* cells are defective in adaptation to growth on glycerol medium when shifted from glucose medium. The severity of the defect to utilize glycerol in *ydr336w* is enhanced when the cells are maintained in glucose medium well into the stationary phase. *YDR332w* belongs to DExH/D super family of RNA helicases targeted to the mitochondria. *YDR332w* orthologs are present in prokaryotes and lower eukaryotes but absent in higher eukaryotes so far sequenced. Yeast cells deleted for *YDR332w* gave rise to two set of colonies that can be differentiated on size on media containing glucose. When *ydr332w* cells were plated on glycerol there were fewer colonies in comparison to wild type cells. Sub-culturing *ydr332w* cells in glucose resulted in changes in the colony size and ability to utilize glycerol as the sole carbon source. The percentage glycerol$^+$ cells among the total viable cells in cultures grown in glucose were found to rapidly decrease. Interestingly the number of *ydr332w*$^+$ cells also decreased when sub-cultured in glucose albeit at a slower rate, indicating that *YDR332w* is essential for mitochondrial function. Our current studies are aimed at understanding the molecular

complex that Ydr332wp and Ydr336wp bind to and whether mitochondrial translation or mito-ribosome assembly are their primary function.

133A. *PEP3* overexpression protects yeast from acid stress by promoting vacuolar biogenesis. ***J. Ding***[1,2], *G. Holzwarth*[2], *S. Bradford*[3], *B. Cooley*[4], *A. Yoshinaga*[5], *J. Patton-Vogt*[4], *H. Abeliovich*[6], *M. Penner*[2], *A. Bakalinsky*[1,2]. 1) Department of Biochemistry & Biophysics, Oregon State University, Corvallis, OR, USA; 2) Department of Food Science & Technology, Oregon State University, Corvallis, OR, USA; 3) Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR, USA; 4) Biological Sciences, Duquesne University, Pittsburgh, PA, USA; 5) Department of Microbiology, Oregon State University, Corvallis, OR, USA; 6) Department of Biochemistry and Food Science, Hebrew University, Rehovot, Israel.
All organisms have the ability to maintain optimal intracellular pH to assure growth and survival. In fungi, two recognized mechanisms that control pH include the plasma membrane proton-pumping ATPase that exports excess protons, and the vacuolar proton-pumping ATPase (V-ATPase) that mediates vacuolar proton uptake. Here, we report that overexpression of *PEP3* which encodes a component of the HOPS and CORVET complexes involved in vacuolar biogenesis, increases tolerance for acid stress in *Saccharomyces cerevisiae*. While we originally detected *PEP3* in a screen for overexpressed genes that conferred acetic acid tolerance, *PEP3*-overexpressing cells also exhibited increased tolerance for sorbic and formic acids. Based on confocal microscopy, *PEP3*-overexpressing cells stained with the vacuolar membrane-specific dye, FM4-64, had more and smaller vacuoles than the wild-type control. By flow cytometry, the stained overexpression mutant was found to exhibit about 3.6-fold more fluorescence than the wild-type control. By quinacrine staining which correlates with vacuolar acidity, the *PEP3*-overexpressing strain was found to have more acidic vacuoles during growth in both the absence and presence of 80 mM acetic acid. During growth in the presence of 80 mM acetic acid, the vacuolar pH of the overexpression strain was 5.38, significantly lower than that of the wild-type control, 6.75. Based on an indirect growth assay, the *PEP3* overexpression strain also exhibited higher V-ATPase activity. We hypothesize that *PEP3* overexpression provides protection from acid stress by increasing the number of vacuoles per cell and hence, proton-sequestering capacity.

134B. Ysp1 homologous proteins participate in responses to membrane stress in *S. cerevisiae* and *S. pombe*. *Vladimir Sirotkin*[1], *Michael James*[1], *Elizabeth Bonarigo*[2], *Gary Franke*[2], *Kelly Hopkins*[2], ***Scott Erdman***[1,2]. 1) Dept of Cell and Developmental Biology, SUNY Upstate School of Medicine, Syracuse, NY; 2) Dept Biol, Syracuse Univ, Syracuse, NY.
The budding yeast protein Ysp1 (Yeast Suicide Protein 1) is implicated in the process of mitochondrial fragmentation in response to stress as it appears that mitochondrial fragmentation in response to either mating pheromone or disruption of calcium homeostasis through the drug amiodarone is Ysp1 dependent. Budding yeast is distinct from other fungi in having a *YSP1* paralogue, *SIP3*. *SIP3* has been implicated in lipid homeostasis in multiple genetic screens. We have found that *sip3* and *ysp1* have very similar phenotypes and genetically interact. Both *sip3* and *ysp1* strains are sensitive to the calcium channel blocker amiodarone, weak acid stress, membrane disrupting agents such as digitonin and amphotericin B, but are resistant to ketoconazole, an antifungal drug that blocks ergosterol biosynthesis. The domain structure of the Ysp1 protein conserved in its homologues in other fungi suggests these proteins to be resident in membranes and a role for these proteins in sensing or generating membrane curvature. These proteins contain both putative BAR and PH domains, as well as transmembrane domains within their carboxy-terminal regions. BAR domains are involved in either sensing or generating membrane curvature and pleckstrin homology (PH) domains target proteins to specific membranes, such as the Golgi or function to integrate cell signaling events. The fission yeast, *S. pombe*, contains one *YSP1* homologue. We created a *ysp1* strain in *S. pombe* and our studies indicate these deletants possess similar phenotypes to the *sip3* and *ysp1* strains in *S. cerevisiae*, including sensitivity to digitonin and amphotericin B and resistance to ketoconazole. Point mutations constructed in the BAR/PH regions and a truncation mutant of the carboxy terminal predicted transmembrane domain region indicate the importance of these domains for function of the protein. *S. pombe ysp1*$^{+}$ is also required for viability in response to membrane stress (amphotericin B). Current studies are directed at investigating how stress conditions influence mitochondrial morphologies in wild type and *ysp1* cells, the localization of the *S. pombe* Ysp1 protein, the importance of conserved residues in its BAR domains, and the role(s) of *ysp1*$^{+}$ in mitochondrial dynamics in response to stress.

135C. Elucidation of the Mitochondrial Protein-Protein Interaction Network. ***Matthew G M Jessulat***[1], *Hiroyuki Aoki*[1], *Zoran Minic*[1], *James Vlasblom*[2], *Sadhna Phanse*[1,3], *Zhaolei Zhang*[3], *Jodi Nunnari*[4], *Mohan Babu*[1]. 1) Department of Chemistry and Biochemistry, University of Regina, Regina, Saskatchewan, Canada; 2) Department of Chemistry, University of Toronto, Toronto, Canada; 3) Banting and Best Department of Medical Research, Donnelly Center, University of Toronto, Toronto, Ontario, Canada; 4) Department of Microbiology and Molecular Genetics, University of California Davis, California, USA.
Affinity-purification/mass spectrometry (AP/MS) methods have need widely used to examine protein-protein interaction (PPI) networks in the model organism *Saccharomyces cerevisiae*, with previous screens elucidating much of the

cytoplasmic and membrane protein interaction network, identifying protein complexes and pathway relationships. However, mitochondrial PPIs are under-represented in these analyses. Here, we describe a method for investigating mitochondrial PPIs via optimized growing conditions for mitochondrial protein expression and optimized protein isolation, as part of an ongoing screen to map the complete mitochondrial PPI network from a curated list of 1179 target proteins. By using non-fermentative media, mitochondria are enriched, improving yield of mitochondrial proteins and avoiding repression of target proteins in standard YPD. A combination of crude mitochondrial isolation and chemical cross-linking of weakly-bound proteins followed by single-step affinity purification allows reduction of non-mitochondrial contamination while still achieving good recovery of prey proteins. Identification of prey proteins is achieved through an Orbitrap Elite mass spectrometer, which improves detection thresholds over previous MS apparatus. Using this system, we have successfully identified known protein interactions for well-established protein complexes such as the MINOS complex, serving to validate the experimental pipeline. Additionally, we have identified novel binding partner interactions and novel complexes for several uncharacterized mitochondrial proteins, potentially allowing for further functional characterization.

136A. Aconitase-MRPL49 fusion protein regulates mitochondrial translation in S. pombe. ***Soo-Jin Jung***, *Youngdae Seo, Jung-Hye Roe.* Seoul National University, Seoul, South Korea.
Aconitase is an enzyme in TCA cycle (Krebs cycle) that converts citrate to isocitrate in mitochondria and bacteria. It contains a [4Fe-4S] cluster which is bound by three cysteins and exposed to solvent, and thus is sensitive to oxidation and iron deficiency. De-metallated aconitases are known to bind nucleic acids. A prominent example is IRP1 (iron-regulatory protein 1) in mammals, which binds to iron-responsive elements (IREs) in the 5 or 3 UTR of specific mRNAs as a demetallated and inactive form of aconitase in the cytoplasm. Several bacterial aconitases were shown to bind RNAs as well. In S. cerevisiae, mitochondrial aconitase (Aco1) is known to bind to mitochondrial DNA. Thus, aconitases are versatile proteins with multiple roles. Schizosaccharomyces pombe contains two genes that encode aconitase. The aco1 gene (SPAC24C9.06c) encodes a protein of 789 aa with a single aconitase domain, whereas the aco2 gene (SPBP4H10.15) encodes a two-domain protein of 918 aa, with aconitase domain fused with mitochondrial ribosomal large subunit protein L21 (MRPL49) at the C terminus. Both aco1 and aco2 genes appear essentially required for the viability of S. pombe. The aco2 gene produces two types of transcripts encoding an aconitase and aconitase-Mrpl49 fusion protein, respectively. Whereas the single aconitase domain protein resides mostly in mitochondria, the fusion protein is localized in the nucleus and cytoplasm as well as in mitochondria. Deletion of Mrpl49 domain made cells nonviable, suggesting its essentiality. Repression of aco2 gene expression inhibited mitochondrial translation, indicating the role of Aco-Mrpl49 fusion protein in this process. Further characterization of Aco2 protein will reveal additional functions other than aconitase enzyme activity.

137B. *MTG3*, a putative GTPase that regulates mitochondrial ribosome function in *Saccharomyces cerevisiae* . ***Upasana Mehra***, *Yash Verma, Kaustuv Datta.* Department of Genetics, University of Delhi, New Delhi, India.
Mitochondria are made up of proteins encoded by the nuclear genome as well as its own genome, thus requiring both cytosolic as well as its own translation apparatus for its biogenesis. Importance of mitochondrial translation apparatus can be estimated with the fact that approximately 25% of the mitochondrial proteome is involved in the establishment and the maintenance of the mitochondrial protein synthesis apparatus and mitochondrial DNA. During evolution, the RNA content of the mitochondrial ribosome has reduced and the protein mass have increased giving rise to numerous mitochondrial specific proteins conserved from lower eukaryotes to humans. Ribosome biogenesis (bacterial/ cytosolic/ mitochondrial) is a complex process which requires ordered association of several ribosomal proteins and rRNAs, aided by a number of assembly factors including GTPases. A number of non-exclusive models have been proposed to account for utilization of energy released upon GTP hydrolysis in (1) recruitment/ removal of individual ribosomal proteins from precursor molecules, (2) alteration of the rRNA structure or (3) conformational changes within the precursor molecule to expose ribosomal protein binding surfaces. Mtg3p, is a nuclear encoded mitochondrial protein that is conserved from yeast to humans and is a member of YawG/YlqF family of circularly permuted GTPases. Members of this family in yeast include Lsg1p, Nog2p and Mtg1p that influence large subunit biogenesis either in cytosol or mitochondria. Deletion of *MTG3* lead to defect in utilization of glycerol as a sole carbon source and aberrant 15S rRNA processing, indicating a role in small subunit biogenesis. My focus is to identify the molecular complex that Mtg3p is associated with, domains of Mtg3p that are essential for its *in vivo* function and the downstream partners/processes that it likely controls. We have shown Mtg3p to be associated with the 37S small subunit of the mitochondrial ribosome in a salt dependent manner. In addition, we have isolated spontaneous suppressors for *yor205c* $^{+}$ and have determined them to be due to second-site mutations in the mitochondrial genome. We are in the process of determining the site of suppressor mutation on the mitochondrial genome. We will also be presenting our studies to determine the site of interaction of Mtg3p on the ribosome and the energy requirements for it.

138C. A genetic screen for suppressors of the age-associated decline of mitochondrial membrane potential. ***NH Thayer***[1,2], *MA Borden*[1], *AL Hughes*[1,3], *D Lindstrom*[1], *FS Vizeacoumar*[4], *C Boone*[4], *DE Gottschling*[1]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 3) Current Location: Biological Chemistry, University of Utah, Salt Lake City, UT; 4) University of Toronto, Toronto, ON.
The budding yeast, *Saccharomyces cerevisiae*, is an excellent model for studying aging because it is a highly tractable, unicellular eukaryote that experiences a short, finite, replicative life span (RLS). Despite these advantages, genome-wide approaches for studying aging in yeast have been hindered by technical difficulties in observing cells of any significant age. Using the previously described Mother Enrichment Program[1] (MEP), our lab has recently been able to identify several age-associated phenotypes. One of these is an age-associated decline in mitochondrial membrane potential[2] (^{mito}). This phenotype was shown to be part of an aging pathway, preceded by a loss of vacuole acidity and ultimately having a role in determining life span. While it has been hypothesized that the loss of vacuole acidity leads to an inability to store amino acids, the exact mechanism by which this affects ^{mito} is unknown. In order to identify regulators of the age-associated decline in ^{mito} and of the aging process in general, we have conducted a genetic screen to identify factors that delay the onset of the reduction in ^{mito}. We have identified several candidates that could be acting between the initial loss of vacuole acidity and ^{mito} or as general regulators of this aging pathway. This approach required the development of a novel assay combining several technologies (MEP, Fluorescence-Activated Cell Sorting, and Barcode Sequencing). This has allowed us to investigate the genetics of age-associated processes on a scale that has not previously been possible. Additionally, we propose that this approach can be extended to study the genetics regulating other known age-associated phenotypes.
[1] Lindstrom, D. L., and Gottschling, D. E. (2009). The mother enrichment program: a genetic system for facile replicative life span analysis in *Saccharomyces cerevisiae*. Genetics 183, 413-22.
[2] Hughes, A. L., & Gottschling, D. E. (2012). An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature 492(7428), 261-265.

139A. A sterol-enriched vacuolar microdomain mediates the stationary phase lipophagy in budding yeast. *Yu-Hsuan Miao, Yi-Shun Chang,* ***Chao-Wen Wang***. Inst Plant & Microbial Biol, Academia Sinica, Taipei, Taiwan.
Stationary phase is a poorly understood physiological state under which cells arrest proliferation and acquire resistance to multiple stresses. Lipid droplets (LDs), organelles specialized for cellular lipid homeostasis, increase the size and number at the onset of the stationary phase. However, little is known about the dynamics of LDs under this condition. Here, we analyze LD distribution during transition to the stationary phase and found that LDs enter the vacuole lumen along with a unique, hydrolase-resistant, membrane during the stage. We show that the LD translocation process requires the core autophagy machinery and a subset of autophagy-related (Atg) proteins involved in selective autophagy. Notably, the process that we term stationary phase lipophagy is mediated through a sterol-enriched vacuolar microdomain whose formation and integrity directly affect LD translocation. Intriguingly, cells defective in the stationary phase lipophagy showed disrupted vacuolar microdomains, implying the LD contents, likely sterol esters, contributes to the maintenance of vacuolar microdomains. Together, we propose a feed forward loop model in which lipophagy stimulates vacuolar microdomain formation, which in turn promotes lipophagy during the stationary phase.

140B. Membrane insertion mechanisms of mitochondrially-encoded proteins by the Oxa1 homolog, Cox18. ***Mei-Yi Zheng***, *Heather L. Fiumera*. Dept. of Biological Sciences, Binghamton University, Binghamton, NY.
The majority of mitochondrially-encoded proteins are inserted and assembled into the inner mitochondrial membrane. Despite the fundamental importance to cell biology, molecular mechanisms of mitochondrial protein biogenesis are not well understood. Oxa1 and Cox18, the only known insertases for mitochondrially-encoded proteins, are structurally similar but act in separate and sequential steps of cytochrome c oxidase assembly. Through an overexpression screen in *S. cerevisiae*, we identified two spontaneously occurring mutants in the 2nd and 4th transmembrane domains of Cox18 that allowed for weak respiration in the absence of Oxa1. Through a protein domain absent on Cox18, Oxa1 normally associates with mitochondrial ribosomes to insert the N-tail domain of the mitochondrially-encoded oxidase subunit, Cox2. Following N-tail insertion, a Cox18-Mss2 complex facilitates post-translational export and assembly of the Cox2 C-tail domain. We found that overexpression of wild type Cox18 in the absence of Oxa1 resulted in the accumulation of N-tail exported, but unassembled, Cox2. Overexpression of the Cox18 mutants did not substantially increase the rate of Cox2 N-tail export, but allowed for the accumulation of processed Cox2 and functional oxidase. Respiration was not improved when ribosomal associations to the site of Cox18-mediated Cox2 assembly were increased by 1) fusing the ribosome-binding domain of Oxa1 to wild type and mutant Cox18 proteins and by 2) overexpression of the membrane-bound mitochondrial ribosome receptor, Mba1. Surprisingly, we found that the Cox18 mutants supported stronger respiratory growth in strains lacking both Mss2 and wild-type Cox18. A co-immunoprecipitation experiment showed that the physical association between wild type Cox18 and Mss2 was dependent upon the presence of Cox2, suggesting that normal mechanisms of Cox2 insertion and assembly are interrupted by these Cox18 mutants. Site-directed mutagenesis experiments of the mutated transmembrane domain positions in Cox18 suggest that a global alteration of the

transmembrane domains may allow Cox18 insertase function to be more permissive. Our results indicate that Oxa1 and Cox18 have mechanistic differences in the recognition and insertion of mitochondrial-encoded membrane proteins, in addition to known differences in substrate-delivery pathways.

141C. Specific -arrestins negatively regulate Saccharomyces cerevisiae pheromone response by down-modulating the G-protein coupled receptor Ste2. ***Christopher Alvaro***[1], *Allyson O'Donnell*[2], *Ann Aindow*[1], *Derek Prosser*[3], *Andrew Augustine*[2], *Aaron Goldman*[4], *Jeff Brodsky*[2], *Martha Cyert*[4], *Beverly Wendland*[3], *Jeremy Thorner*[1]. 1) Div. of Biochemistry, Biophysics and Structural Biology, Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA; 2) Dept. of Cell Biology, Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA; 3) Dept. of Biology, Johns Hopkins Univ., Baltimore, MD; 4) Dept. of Biology, Stanford Univ., Stanford, CA.
G-protein-coupled receptors (GPCRs) are integral membrane proteins that initiate responses to extracellular stimuli by mediating ligand-dependent activation of cognate heterotrimeric G-proteins. In yeast, occupancy of GPCR Ste2 by peptide pheromone -factor initiates signaling by releasing a stimulatory G complex (Ste4-Ste18) from its inhibitory G subunit (Gpa1). Prolonged pathway stimulation is detrimental, and feedback mechanisms have evolved that act at the receptor level to limit the duration of signaling and stimulate recovery from pheromone-induced G1 arrest, including up-regulation of the expression of an -factor-degrading protease (Bar1), a regulator of G-protein signaling protein (Sst2) that stimulates Gpa1-GTP hydrolysis, and Gpa1 itself. Ste2 is also down-regulated by endocytosis, both constitutive and ligand-induced. Ste2 internalization requires its phosphorylation and subsequent ubiquitinylation by the HECT-domain containing ubiquitin ligase (Rsp5). Here we demonstrate that three different members of the -arrestin family of trafficking adaptors (Ldb19/Art1, Rod1/Art4 and Rog3/Art7) contribute to Ste2 desensitization and internalization, and do so by discrete mechanisms. Cells lacking these -arrestins show an increase in pheromone sensitivity and display more Ste2-GFP at the PM. Genetic and biochemical analysis shows that Ldb19 and Rod1 recruit Rsp5 to Ste2 via PPxY motifs in their C-terminal regions. By contrast, the arrestin-fold domain at the N-teminus of Rog3 is sufficient to promote adaptation, indicating that it can impose an additional Rsp5-independent mechanism of negative regulation. Finally, we show that phosphorylation regulates Rod1 function in the mating pathway; its function is inactivated by the protein kinase Snf1 and calcineurin-dependent dephosphorylation of Rod1 is required for its role in down-regulation of Ste2-initiated signaling. Elimination of the Snf1 phosphorylation sites in Rod1 (by mutation to Ala) renders Rod1 hyperactive in promoting adaptation to pheromone.

142A. Stress conditions promote Gap1 permease ubiquitylation and downregulation via the arrestin-like Bul and Aly proteins. *M. Crapeau, A. Merhi,* ***B. Andre***. IBMM, Free University of Brussels, Gosselies, Belgium.
Gap1, the yeast general amino acid permease, is a convenient model for studying how the intracellular trafficking of membrane transporters is regulated. Present at the plasma membrane under poor nitrogen supply conditions, it undergoes ubiquitylation, endocytosis and degradation upon stimulation of the TORC1 kinase complex following an increase of internal amino acids, eg. after ammonium uptake. Stimulated TORC1 phosphoinhibits the Npr1 kinase resulting in activation by dephosphorylation of the arrestin-like Bul1 and Bul2 adaptors, which recruit the Rsp5 ubiquitin ligase to Gap1. We now report that Gap1 is also downregulated when cells are treated with the TORC1 inhibitor rapamycin or transferred to various stress conditions, while lack of Tco89 subunit of TORC1 provokes constitutive Gap1 downregulation. The Bul1 and Bul2 as well as Aly1 and Aly2 arrestin-like adaptors promote the Gap1 ubiquitylation inducing this downregulation without undergoing dephosphorylation. Furthermore, they act via C-terminal regions of Gap1 uninvolved in ubiquitylation in response to internal amino acids. While the Buls mediate Gap1 ubiquitylation on two possible lysines, K9 or K16, the Alys only promote Gap1 ubiquitylation of its K16 residue. This downregulation pathway, targeting other permeases as well, likely allows cells facing adverse conditions to retrieve amino acids from permease degradation.

143B. The protein quality control machinery regulates its misassembled proteasome subunits, and distinguishes them from proteasome storage granules. ***S. Ben-Aroya****, L. Peters, O. Yogev, R. Hazan.* Bar-Ilan University, Ramat-Gan, Israel.
In eukaryotes, the ubiquitin proteasome system (UPS) plays a vital role in protein quality control (PQC), by selectively targeting misfolded proteins for degradation. While the assembly of the proteasome can be naturally impaired by many factors, the regulatory pathways that mediate the sorting and elimination of misfolded proteasomal subunits is poorly understood. Our study reveals how the misassembled proteasome is controlled by the PQC machinery. We found that among the multilayered quality control mechanisms, UPS mediated degradation of its own dysfunctional subunits is the favored pathway. We also demonstrated that the Hsp42 chaperone could mediate an alternative pathway, the accumulation of these subunits in cytoprotective compartments, and also distinguish them from additional, and probably functional, proteasome aggregates that are formed upon carbon depletion. These results highlight the importance of removing potentially cytotoxic protein aggregates, despite the high cost of diverting essential protein products. Thus, we show that proteasome homeostasis is controlled through probing the quality of proteasome aggregates, and the interplay between UPS mediated degradation or their sorting into distinct cellular compartments.

144C. Cdc48-Shp1 chaperone promotes structural integrity of protein phosphatase 1 holoenzyme. ***You-Liang Cheng***[1,2], *Rey-Huei Chen*[1]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan.
Protein phosphatase 1 (PP1) controls many aspects of cell physiology, which depends on its correct targeting in the cell. Nuclear localization of Glc7, the catalytic subunit of PP1 in budding yeast, requires Cdc48 and its adaptor Shp1 through unknown mechanism. In this report, we show that mutations in SHP1 cause misfolding of Glc7 that co-aggregates with Hsp104 and Hsp42 chaperones and requires the proteasome for clearance. Mutation or depletion of PP1 regulatory subunits Sds22 and Ypi1 that are involved in nuclear targeting of Glc7 also produce Glc7 aggregates, indicating that association with regulatory subunits stabilizes Glc7 conformation. Use of a substrate-trap Cdc48QQ mutant reveals that Glc7-Sds22-Ypi1 transiently associates with and is the major target of Cdc48-Shp1. Furthermore, Cdc48-Shp1 binds PP1-like phosphatases Ppz2 and Ppq1 and maintains their conformational integrity. Our study suggests that Cdc48-Shp1 functions as a molecular chaperone for proper folding and assembly of PP1 complex.

145A. The Yeast Biogenesis of Lysosome Related Organelle (BLOC) Complex interacts with ESCRT to regulate endosomal trafficking. ***Lauren E Dalton***[1], *Matthew Jessulat*[2], *Viktor Deineko*[2], *Jeffery Tong*[1], *Mohan Babu*[2], *Elizabeth Conibear*[1]. 1) Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada; 2) Department of Biochemistry, Research and Innovation Centre, University of Regina, Regina, Saskatchewan, Canada.
Endosomal sorting is a process that recognizes proteins and targets them for degradation or recycling to another destination. In mammals, the eight subunits of the Biogenesis of Lysosome Related Organelles Complex 1 (BLOC-1) interact with the AP-3 adaptor complex to transport specific proteins from endosomes to lysosome-related organelles (LROs). Defects in this pathway are a cause of Hermansky-Pudlak Syndrome. A recent bioinformatics analysis uncovered an evolutionary relationship between mammalian BLOC-1 and three of the six subunits of an uncharacterized yeast complex referred to as BLOC. We find the yeast BLOC complex is dispensable for AP-3 mediated sorting and instead interacts genetically, physically and functionally with the Endosomal Sorting Complex Required for Transport (ESCRT) complex. Each of the 6 BLOC subunits binds the ESCRT-0 subunit Vps27 and co-localizes with ESCRT proteins at late endosomes. Whereas mammalian BLOC-1 interacts with adaptor protein complex 3 (AP-3) to target proteins to LROs, yeast AP-3 pathway is not affected by loss of the BLOC complex. Instead, BLOC mutants alter the trafficking of the manganese transporter Smf1 at endosomes. This work suggests that the yeast BLOC complex cooperates with the ESCRT complex at endosomes to sort specific cargoes for degradation in the vacuole.

146B. Identifying new protein trafficking networks using evolutionary rate covariation (ERC). ***Zelia A. Ferreira***[1], *Allyson F. O'Donnell*[2], *Nathan L. Clark*[1]. 1) Computational and Systems Biology, University of Pittsburgh, 15260, Pittsburgh, PA; 2) Dept. of Cell Biology, Univ. of Pittsburgh, 15260, Pittsburgh, PA.
Arrestins regulate protein trafficking and cell signaling in response to environmental changes. In *S. cerevisiae*, -arrestins control endocytosis of membrane cargo proteins by selectively recruiting the ubiquitin ligase Rsp5, which ubiquitinates the cargo to promote internalization. By controlling selective recruitment of Rsp5 to membrane transporters, -arrestins play an important role in shaping the protein content at the plasma membrane. However, identifying -arrestin targets is a challenging task due to the inherent difficulties of identifying interactors with membrane proteins and because -arrestins associate transiently with their membrane cargo. To overcome these problems, we employed a novel computational approach, Evolutionary Rate Covariation (ERC), to identify new cargos regulated by -arrestins. ERC is a sequence-based signature that identifies genes with similar evolutionary histories, and has been employed to discover novel pathway genes in *Drosophila* and in human genetic diseases. The basis of the ERC signature is that functionally related genes experience similar environmental changes and hence they tend to have evolutionary rates that statistically covary. Using ERC, we analyzed the rate covariation of -arrestins with all genes over a phylogeny of 18 yeast species. Among the top scoring genes were previously confirmed -arrestin cargos. Therefore, we hypothesized that other top hits, including transporters and signaling receptors, are -arrestin cargos as well. Moreover, among the top ERC scores there was a strong enrichment for plasma membrane proteins, which is consistent with -arrestin function. We validated a subset of these new -arrestin cargos by assaying the localization of GFP-tagged cargo proteins in cells lacking specific -arrestins. To date we confirmed that three cargos are regulated by -arrestins as predicted in our ERC analysis. Specifically, the -arrestin Ldb19 regulates the trafficking of transporters from a multidrug resistance family. Current efforts focus on using ERC to infer cargos for -arrestins in yeast and mammals to be validated in vivo. Overall, ERC has revealed potential to guide the protein trafficking field by identifying new functional relationships between trafficking machinery and their cargos.

147C. Hsp31 is a Stress-response Chaperone that Prevents -Synuclein Aggregation. *Chai-jui Tsai*[1], *Kiran Aslam*[1], *Holli Drendel*[2], *Josephat Asiago*[1], *Kourtney Fultz*[1], *Jean-Christophe Rochet*[1], ***Tony Hazbun***[1]. 1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN; 2) Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN.
The *Saccharomyces cerevisiae* heat shock protein Hsp31 is a stress-inducible homodimeric protein belonging to the DJ-

1/ThiJ/PfpI superfamily. Here we show that substoichiometric concentrations of Hsp31 can abrogate aggregation of a broad array of substrates *in vitro*. Hsp31 shares a conserved target substrate, -synuclein (Syn), with human DJ-1. We demonstrate that Hsp31 is able to suppress the *in vitro* fibrillization of Syn, aggregation of citrate synthase and insulin. Chaperone activity was also observed *in vivo* because constitutive overexpression of Hsp31 reduced the incidence of Syn inclusions and yeast cells are rescued from Syn-generated proteotoxicity upon Hsp31 overexpression. Moreover, we confirmed an observation that Hsp31 is induced by H_2O_2, and additionally demonstrate increased expression in the diauxic phase of normal growth conditions and in cells under Syn mediated proteotoxic stress. We also demonstrate reduced aggregation of the Sup35 prion domain, PrD-Sup35, as visualized by fluorescent protein fusions. Localization studies established that Hsp31 does not mutually localize with prion aggregates, and the prevention of detectable Syn fibrils from in vitro studies, indicate that Hsp31 acts on its substrates prior to the formation of the large aggregates. These studies establish that the protective role of Hsp31 against cellular stress can be achieved by chaperone activity on a wide spectrum of misfolded proteins and in particular, Syn.

148A. Degeneracy of parameters underlying partitioning of macromolecular content upon yeast cell division. *Ali Kinkhabwala[2], Anton Khmelinskii[1], **Michael Knop**[1].* 1) ZMBH, University of Heidelberg, Heidelberg, Germany; 2) Max Planck Institut of molecular Physiology, Dortmund, Germany.
Asymmetric cell division, whereby a parent cell generates two sibling cells with unequal content and thereby distinct fates, is central to cell differentiation, organism development and ageing. Unequal partitioning of the macromolecular content of the parent cell which includes proteins, DNA, RNA, large proteinaceous assemblies and organelles can be achieved by both passive (e.g. diffusion, localized retention sites) and active (e.g. motor-driven transport) processes operating in the presence of external polarity cues, internal asymmetries, spontaneous symmetry breaking, or stochastic effects. However, the quantitative contribution of different processes to the partitioning of macromolecular content is difficult to evaluate. To address this, we developed a formal model that allows rapid and quantitative assessment of partitioning as a function of various parameters including passive (diffusion-limited) retention, active transport, asymmetric binding and assembly formation. Application of our model to the budding yeast Saccharomyces cerevisiae reveals important quantitative degeneracies among the parameters that govern the asymmetric division of its various cellular structures.

149B. Vps13 plays a role at the early and late endosomes. ***Kathleen L Kolehmainen**[1,2], Elizabeth Conibear[2].* 1) Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; 2) Centre for Molecular Medicine and Therapeutics, Vancouver, British Columbia, Canada.
Chorea-acanthocytosis and Cohens syndrome are two rare genetic diseases caused by mutations in *VPS13A* and *VPS13B*, respectively. Both genes encode for proteins however the role of these proteins is unknown. Their homology to *Saccharomyces cerevisiae* Vps13 suggests that they may be involved in protein trafficking. Therefore delving further into the role and localization of Vps13 will provide important insights into the function of the human homologs and help elucidate the mechanism of disease. Previous studies have found that a *vps13* mutant secretes the vacuolar enzyme carboxypetidase Y, suggesting that Vps13 mediates recycling from the late endosome. To determine if Vps13 is also required for sorting at early endosomes, we examined the localization of a Snc1-Suc2 construct using an enzymatic assay. In a wild-type strain this construct localizes to the plasma membrane where it can hydrolyze sucrose to produce a brown pigment. In a *vps13* strain, no formation of brown pigment was seen suggesting that recycling from the early endosome to Golgi was disrupted. Using fluorescence microscopy, Vps13-GFP was found to co-localize with both early and late endosomal markers. Localization of Vps13 at the early and late endosomes may be through interaction with the endosomal membrane or mediated by an adaptor protein. A PH domain-like region was predicted at the C-terminus of Vps13 using Phyre2 modelling. PH domains are able to bind phosphoinositide species present in organelle membranes. Alignment and modeling of the C-termini of the human VPS13 family showed that VPS13A but not VPS13B has a potential PH domain-like region at its C-terminus. Currently, the role of adaptor proteins in Vps13 localization is being investigated and initial results suggest that both an adaptor protein and the PH domain-like region of Vps13 interact with the endosomal membrane to localize Vps13.

150C. Dissecting natural variation in yeast to identify genetic modifiers of protein aggregation. ***Theodore W. Peters**, Christopher S. Nelson, Akos A. Grencser, Kathleen J. Dumas, Gordon J. Lithgow, Rachel B. Brem, Robert E. Hughes.* Buck Institute for Research on Aging Novato, CA.
Defects in protein homeostasis are increasingly being implicated in the pathogenesis of a diverse range of late onset human diseases. Aggregates of proteinopathy factors can adopt multiple distinct conformers, analogous to the so-called strains of infectious prions. Aggregation of non-pathogenic protein factors in response to cellular stress suggests a broader role for protein aggregates in maintenance of proteostasis. The genes that control the balance, formation and morphologies of protein aggregates remain poorly understood, in part because assays of aggregation are not well-suited to high-throughput screen methods. To meet this challenge, and uncover the molecules and gene networks that underlie protein aggregation traits, we have developed a genetic-mapping approach to dissect the variation in protein homeostasis control across wild

Saccharomyces cerevisiae individuals. We expressed a GFP-tagged, non-toxic mutant huntingtin (Q75) fragment in the progeny from a cross between a pair of genetically distinct yeast strains and scored the number of fluorescent aggregates that form in each strain. Aggregate load differed by 2-fold across strain backgrounds, and linkage mapping of these quantitative traits mapped loci containing compelling and testable candidate determinants of protein aggregation. Ongoing experiments seek to validate these genes for a causal role in huntingtin aggregation and the effect on the proteostatic network, in yeast and in a *C. elegans* model. We anticipate that the determinants of protein aggregation identified in our work will shed new light on mechanisms of proteostasis control during aging and disease.

151A. Impairment of translocon-associated protein degradation under conditions of endoplasmic reticulum stress. ***Eric M. Rubenstein***[1], *Mark Hochstrasser*[2]. 1) Department of Biology, Ball State University, Muncie, IN; 2) Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.
Misfolded or otherwise aberrant proteins at the endoplasmic reticulum (ER) are ubiquitylated and proteasomally destroyed in the process of ER-Associated Degradation (ERAD). With a few exceptions, the *Saccharomyces cerevisiae* ubiquitin ligases Hrd1 and Doa10 recognize unique classes of ERAD substrates based on the location of substrate lesions. Hrd1 ubiquitylates proteins with degrons (degradation signals) in the ER lumen (ERAD-L) or within membrane-spanning segments (ERAD-M), while Doa10 targets proteins with degrons in the cytosol (ERAD-C). Until recently, it was not clear how (or if) proteins that aberrantly or persistently engage the translocon are targeted for proteasomal destruction. Our recent work indicated that such proteins are targeted for degradation by Hrd1 in a previously uncharacterized process provisionally named ERAD-T (translocon). Post-translational modification and Hrd1-mediated degradation of ERAD-T substrates are markedly impaired under conditions known to elevate the burden of unfolded proteins in the ER and cause ER stress. This effect is at least partially specific to ERAD-T, as degradation of the model ERAD-M substrate 6myc-Hmg2 is minimally affected by ER stress. Neither the canonical unfolded protein response (UPR) nor the ER stress surveillance (ERSU) pathways are required for ERAD-T in the absence of stress or for stabilization of ERAD-T substrates under stress conditions. These data suggest that components of a novel ER-stress-sensing mechanism may play a role in degradation of proteins that aberrantly engage the translocon.

152B. Substrate-induced ubiquitylation and endocytosis of Gap1 and Can1 permeases: role of arrestin-like proteins. ***Elie Saliba***[1], *Kassem Ghaddar*[1], *Ahmad Merhi*[1], *Eva-Maria Krammer*[2], *Martine Prévost*[2], *Bruno André*[1]. 1) Molecular Physiology of the Cell, Université Libre de Bruxelles, IBMM, 6041 Gosselies, Belgium; 2) Structure and Function of Biological Membranes, Université Libre de Bruxelles, Campus Plaine, 1050 Brussels, Belgium.
Many yeast plasma membrane proteins are downregulated by ubiquitylation, endocytosis, and targeting to the vacuole, in response to various stimuli. The yeast general amino acid permease, Gap1, is ubiquitylated and downregulated in response to intracellular accumulation of amino acids. This internal amino-acid signal activates the TORC1 kinase complex that in turn stimulates, via control of the Npr1 kinase and Sit4 phosphatase, the arrestin-like Bul adaptors mediating ubiquitylation of Gap1 by the Rsp5 ubiquitin ligase. We now report that Gap1 and the arginine-specific permease, Can1, undergo ubiquitin-dependent downregulation in response to their substrates, and that this downregulation is not due to intracellular accumulation of the transported amino acids but to transport catalysis itself. Although this endocytosis requires proper recognition of the amino acid by the substrate-binding pocket of the permease, transport is not essential, as an inactive Can1 mutant undergoes normal arginine-induced endocytosis. Substrate-mediated downregulation of Gap1 and Can1 requires the arrestin-like Bul1-2 and Art1 adaptors of Rsp5, respectively. Further observations support the model that the substrate-induced conformational transition inducing endocytosis involves remodeling of cytosolic regions of the permeases, thereby promoting recognition by arrestin-like adaptors able to recruit the Rsp5 ubiquitin ligase. Similar mechanisms might control the abundance of many other plasma membrane transporters according to the external concentrations of their substrates.

153C. Mitochondrial dynamics and the selectivity of mitophagic processes. ***Kobi J. Simpson-Lavy***, *Hagai Abeliovich.* Department of Biochemistry and Food Science, Hebrew University of Jerusalem, Rehovot, Israel.
The autophagic degradation of mitochondria, or mitophagy, is an important housekeeping function in eukaryotic cells. Defects in mitophagy occur are found in degenerative disorders such as Parkinsons and Huntingtons diseases, and decreases in the levels of mitophagy correlate with aging phenomena. An important research goal is to achieve an understanding of the quality control mechanism(s) that specifically cull defective mitochondrial components or compartments, during mitophagy. Specifically, it is not known whether a small cohort of defective molecules will cause the ablation of an entire compartment, or whether an active process segregates defective molecules from functional ones and allows a more efficient culling mechanism. In this study we have combined a global proteomic analysis with a molecular genetic and cell biology approach, to determine whether such a segregation process occurs in yeast mitochondria. We report that different mitochondrial matrix proteins undergo mitophagic degradation at distinctly different rates, supporting the active segregation hypothesis. These differential degradation rates depend on mitochondrial dynamics, suggesting a mechanism that couples a weak physical segregation with mitochondrial fission and fusion to

achieve a distillation-like effect. In agreement with this interpretation, the differential rates of mitophagy that we observed for different mitochondrial matrix proteins strongly correlate with the degree of physical segregation observed for these proteins, within the matrix.

154A. A direct role of HRD3 in ER associated degradation (ERAD). ***Nidhi Vashistha***[1], *Sarah Carroll*[2], *Randolph Hampton*[1]. 1) Cell and Developmental Biology, University of California San Diego, La Jolla, CA; 2) University of Washington, Seattle,WA.
The HRD pathway is a conserved ubiquitin-dependent route of ERAD for misfolded proteins. The HRD complex includes the Hrd1 a RING-H2 E3 ubiquitin ligase and a variety of other cofactors including Usa1 and Hrd3. Usa1 promote self-degradation of Hrd1 while Hrd3 is essential for its stability. Loss of Hrd3 in a hrd3 null strain results in profound lowering of the Hrd1 ligase due to its unrestricted self degradation. Since Hrd3 is required for the stability of Hrd1, and direct involvement in ERAD has never been unambiguously tested by use of the simple hrd3 null. Our earlier observation that deletion of the UBL domain of Usa1 stabilized Hrd1 independent of Hrd3 has enabled us for the first time to address the direct role of Hrd3 in ERAD. Cells harboring Usa1 with the Ubl domain removed (Usa1-UBL) are fully competent for ERAD of all HRD pathway substrates examined. However, in this genetic background loss of Hrd3 no longer causes the profound decrease in Hrd1. Thus, the role on Hrd3 in ERAD independent of Hrd1 stability effects can be evaluated in the Usa1-UBL background. In Usa1-UBL cells when Hrd3 was deleted the degradation of prototype ERAD-M and ERAD-L substrates such as Hmg2, Sec61-2, Pdr5* and CPY* was completely blocked despite Hrd1 remaining stable. Furthermore, we also observed that the ubiquitination of the ERAD-M substrates Hmg2 and Pdr5* was similarly directly dependent on the presence of Hrd3. The studies with Usa1-UBL indicate that Hrd3 has a direct role in ERAD, and specifically it is required for the efficient ligase action of Hrd1. To further examine this idea of direct effect we compared the degradation of the ERAD substrates in presence and absence of stoichiometric amounts of Hrd3 when Hrd1 is overexpressed. As previously observed by us and other laboratories, overexpression of Hrd1 accelerates the degradation of a variety of ERAD substrates, and this rate-limiting effect does not require Hrd3. However further addition of Hrd3 significantly and synergistically accelerate ERAD of both ERAD-M and ERAD-L substrates. Thus, by this independent measure, Hrd3 also demonstrates direct and positive role in HRD-dependent ERAD. Although a direct role of Hrd3 in ERAD-L is consistent with its recruitment of several lumenal mediators, the importance of Hrd3 in ERAD-M indicates a direct and novel mode of Hrd1 activation or membrane substrate recruitment.

155B. The alternate clathrin adaptor complex subunit Apm2 works with Ima1p in a distinct protein transport pathway. ***Shawn T. Whitfield***[1,2], *Helen E. Burston*[2], *Nandini Raghuram*[2], *Elizabeth Conibear*[2]. 1) Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; 2) Centre for Molecular Medicine and Therapeutics, Vancouver, British Columbia, Canada.
Heterotetrameric adaptor protein (AP) complexes recognize cargo proteins for inclusion into clathrin-covered vesicles in post-Golgi transport, and are extremely important for correct localization of a variety of proteins necessary for proper cell functioning. The common view is that yeast have three distinct AP complexes (AP-1, AP-2 and AP-3). AP-1 can include one of two medium chains, Apm1p or Apm2p, to form the full complex but these alternate forms of AP-1 are currently thought to be redundant. We investigated the role of Apm2p, an alternate medium subunit of the AP-1 complex, and suggest that it is not simply a functionally redundant version of the main AP-1 medium subunit Apm1p. Drug sensitivity screens showed drug-specific phenotypes distinguishing *apm2* from *apm1* strains. Furthermore, bioinformatics approaches identified not only Apm2p but also the protein product of an uncharacterized gene that we have named Ima1p (Interacts with medium adaptin 1) as central components of a distinct network. Apm2p was shown to co-immunoprecipitate with Ima1p and yeast two-hybrid studies mapped the interaction to the Apm2p C-terminal domain and the Ima1p N-terminal domain. Notably, this interaction was outside of the tyrosine cargo binding domain of Apm2p, suggesting that Ima1p is not simply trafficked by Apm2p but instead has a regulatory role. Bioinformatics indicate that Ima1p is a member of a conserved family of / hydrolases and may act as a lipase. In all, these results point to a distinct and important role for Apm2p in protein trafficking and for Ima1p in its functioning.

156C. Non-Preferred Carbon Source Utilization Induces Snf1p- and ER Stress-Dependent Activation of the High Osmolarity Glycerol (HOG) Pathway. ***Hema Adhikari***, *Paul Cullen.* Department of Biological Sciences at SUNY-Buffalo, NY 14260-1300.
Evolutionarily conserved mitogen activated protein kinase (MAPK) pathways regulate the response to different stimuli. In Saccharomyces cerevisiae, non-preferred carbon sources, like galactose, induce an ERK-type MAPK pathway that regulates filamentous growth. The filamentous growth pathway shares components with a p38-type MAPK pathway called the High Osmolarity Glycerol (HOG) response pathway, which regulates the response to changes in osmolarity. Here we show that non-preferred carbon sources activate the HOG pathway. HOG pathway activation in this context required the major nutrient regulatory AMP-dependent kinase (AMPK) Snf1p and an intact respiratory chain. The HOG pathway was also induced by protein glycosylation deficiency, which like galactose is also a major inducer of the filamentous growth

pathway. Interestingly, galactose triggered the activation of the unfolded protein response (UPR) and required the unfolded protein response regulator Ire1p, which is a newly established regulator of the HOG pathway. Thus, galactose and glycosylation deficiency concomitantly induces activation of the filamentous growth and HOG pathways. Remarkably, the two MAPK pathways modulated each others activity in these contexts. This cross-modulation optimized the cellular response to biofilm/mat formation and invasive growth. Thus, a new regulatory axis links the utilization of poor carbon source to ER stress, which regulates opposing (ERK and p38 MAPK) pathways by a mechanism that involves the AMPK Snf1p. Analogous pathways (CaHOG and Cek1p) in the fungal pathogen Candida albicans showed similar responses, indicating that this axis is evolutionarily conserved.

157A. ER Stress Stimulates Mucin Receptor Signaling From the Secretory Pathway. ***Hema Adhikari***[1*], *Nadia Vadaie*[1], *Jacky Chow*[1], *Christopher Stefan*[2], *Jason MacGurn*[2], *Paul Cullen*[1]. 1) Department of Biological Sciences at SUNY-Buffalo, 14260-1300; 2) Weill Institute for Cell and Molecular Biology & Department of Molecular Biology and Genetics Cornell University Ithaca NY 14853-7202.
Cell-surface receptors can be activated at the plasma membrane by binding to extracellular stimuli. Some receptors retain their activity in other compartments, like the endosome, after internalization from the plasma membrane. Here, we report a new mechanism for the activation of receptors that can be initiated in the secretory pathway. We show that the signaling mucin Msb2p, which is activated by proteolytic processing and regulates a Cdc42p-dependent MAPK pathway called the filamentous growth pathway, was under-glycosylated in response to carbon limitation, which led to processing of the protein by a protein quality control mechanism involving the unfolded protein response (UPR). As a result, Msb2p was processed in the secretory pathway and could initiate MAPK signaling in trafficking mutants defective for the delivery of plasma membrane cargoes. Our study brings to light a new activation mechanism for receptors in the secretory pathway. Modulating receptor activity by quality control pathways in the endomembrane system represents a new and general way to regulate receptor activity.

158B. Quantifying the effect of coding sequence variation in human orthologs of *Saccharomyces cerevisiae* Bim1 on the pheromone response. ***D. Britain***[1,2,3], *B. Sands*[1], *W. Peria*[1], *G. Pesce*[4], *R. Brent*[1,2,5]. 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Department of Bioengineering, University of Washington, Seattle, WA; 3) Department of Biochemistry, University of Washington, Seattle, WA; 4) The Molecular Sciences Institute, Berkeley, CA; 5) Department of Genome Sciences, University of Washington, Seattle, WA.
The yeast Bim1 protein binds the plus ends of cytoplasmic microtubules. In dividing cells, Bim1 helps cytoplasmic microtubules bridge between the cell nucleus and the cell membrane by attaching the microtubule plus ends to the site of polarized cell growth. This microtubule bridge helps set the correct plane of nuclear division and cell division. During the pheromone response, the microtubule bridge connects the nucleus and the site on the cell membrane where signaling molecules such as the receptor, Ste2, accumulate. This site will later become the tip of the mating projection. As quantified by reporter gene expression in single cells (Colman-Lerner et al. 2005), deletion of the *bim1* gene causes increased variation in pheromone signaling and impaired polarization decisions due to impaired attachment of the microtubule to the signaling site (Pesce et al., in preparation). The Bim1 protein has three mammalian orthologs: MAPRE1, MAPRE2, and MAPRE3. Recent work from the 1000 Genomes Project, the Exome Sequencing Project, and from other efforts has revealed numerous coding sequence variants in these genes present, usually as heterozygotes, in the current human population. I designed and constructed strains that lacked native Bim1 but that expressed different Bim1 proteins mutated at residues conserved among Bim1 and MAPRE proteins, and whose mutations corresponded to MAPRE coding sequence variants in the human population. At least one humanized *bim1*- allelic variant increased variation in signaling and may do so when heterozygous with wild-type *BIM1*+;. This work raises the possibility that some coding sequence variants of human microtubule binding proteins might increase variation in cell signaling and negatively impact proper cell polarization during embryonic development and in maintenance of adult tissues. It also shows that quantification of subtle signaling phenotypes in humanized yeast can suggest hypotheses about how specific allelic variants might contribute to the burden of human disease.
Colman-Lerner, A., Gordon, A., Serra, E., Chin, T., Resnekov, O., Endy, D., Pesce, G. and Brent, R. (2005) Regulated cell-to-cell variation in a cell fate decision system. Nature. 437, 699-706.

159C. Global Analysis of Filamentous Growth Pathway Regulators in Yeast. *Colin A. Chavel*, ***Lauren M. Caccamise***, *Boyang Li, Paul J. Cullen.* University at Buffalo, SUNY, Buffalo, NY.
Cell differentiation requires multiple signaling pathways to act in concert to produce a specialized cell type. *Saccharomyces cerevisiae* undergoes filamentous growth, a cell-differentiation response to nutrient limitation. Filamentous growth requires the action of multiple pathways, including a mitogen activated protein kinase (MAPK) pathway. Our lab and other labs have shown that several of the pathways that regulate filamentous growth also regulate the filamentous growth MAPK pathway. To identify new regulatory connections to the MAPK pathway, a genetic screen was performed using a nonessential deletion collection in the filamentous background. Together with direct testing, we

validated known regulators of the MAPK pathway [including Ras2p-cAMP-Tpk2p-Flo8p-Sfl1p, the retrograde pathway, phospholipid biosynthesis control components (Nte1p and Opi1p), pH sensing Rim101 pathway, Elongator (ELP), and Rpd3p(L)] and uncovered new connections [including members of the phosphate sensing pathway, prefoldin, and chromatin remodeling protein Gcn5p (SLIK)]. Breaking the regulatory connections led to an uncoordinated response. Many of the pathways also regulated each other, which indicates that the pathways that control filamentous growth function in an integrated network. Our findings provide a template for how MAPK-dependent differentiation is regulated that may extend to higher eukaryotes.

160A. Structure and function of the TORC1-PKA signaling network in budding yeast. *James Hughes Hallett, Joe Kunkel, Xiangxia Luo,* ***Andrew Capaldi***. University of Arizona, Tucson, AZ.
To thrive when conditions are favorable, and survive when they are stressful, cells must set their growth rate based on the level and combination of numerous intracellular and extracellular stimuli. It is now well established that this information processing is carried out (to a large degree) by the TORC1 and ras/PKA signaling pathways. However, it remains unclear how these pathways function together as a control system. To address this question, we constructed a detailed model of the TORC1-PKA signaling network in budding yeast by following signaling and gene expression in strains with key proteins knocked out (or blocked by chemical inhibition), in nine stress and starvation conditions. Together our data show that: (1) The TORC1 pathway acts a sophisticated feedback control circuit, activating different proteins and genes, depending on the needs of the cell. Specifically, when cells are starved for glucose (energy), AMP-activated protein kinase (AMPK) inhibits the TORC1 pathway so that cell growth halts and no TORC1 dependent metabolic reprogramming can occur. By contrast, in the presence of energy, the TORC1 pathway can exist in three different states. First, in rich medium, TORC1 is active and drives protein and ribosome synthesis through the Sch9 branch of the pathway. Second, when cells are starved for nitrogen/amino acids, the pathway enters the previously identified OFF state where the Rag and Rho1 GTPases bind TORC1 to block cell growth and activate TORC1-PP2A dependent amino acid synthesis. Third, when cells are treated with noxious agents, such as high salt, TORC1 dependent cell growth is inhibited while the cell mounts a stress response. (2) The PKA pathway acts as a glucose dependent feedforward control circuit. Specifically, when cells are transferred into medium containing glucose, the PKA kinases act in parallel with TORC1 to boost the expression of 99% of the TORC1 dependent genes. However, once the cells reach the log growth phase in glucose, PKA signaling decreases dramatically so that TORC1 acts alone to control cell growth. At the same time, low level signals through PKA stabilize the transcriptional repressors Dot6/Tod6, proteins inhibited by TORC1 and PKA, so that removal of glucose now triggers a rapid decrease in protein and ribosome synthesis. Again, once this transition is over, TORC1 acts alone to control cell growth (due to degradation of Dot6/Tod6). Thus, the cell growth control system in yeast acts in a similar way to many man-made control systems, with a feedback circuit setting the precise output level based on internal changes, and a feedforward controller driving the system to new output levels in the face of large external changes.

161B. The Cdc42-Interacting Protein Bem4 Regulates the Filamentous Growth Pathway. ***Colin A. Chavel***[1]*, Andrew Pitoniak*[1]*, Jeremy Smith*[1]*, Diawoye Camera*[1]*, Jacky Chow*[1]*, Sheelarani Karunanithi*[1]*, Ken Wolfe*[2]*, Paul Cullen*[1]. 1) University at Buffalo, SUNY, 337 Cooke Hall, North Campus, Buffalo, NY 14260; 2) Smurfit Institute of Genetics,Trinity College, University of Dublin, Dublin 2, Ireland.
Rho GTPases such as the ubiquitous regulator Cdc42p control cell polarity and signal transduction pathways including mitogen activated protein kinase (MAPK) pathways. How Cdc42p and other general factors are directed to a specific pathway remains an open question. We show here that the Cdc42p-interacting protein Bem4p regulates the MAPK pathway that controls filamentous growth in yeast. Bem4p associated with the plasma-membrane adaptor Sho1p in vivo and the guanine nucleotide exchange factor (GEF) for Cdc42p, Cdc24p, *in vitro* and *in vivo*. *In vitro* pulldown studies with truncated versions of Cdc24p indicate that Bem4p interacts with PH domain of Cdc24p, which may activate the GEF. Bem4p regulated Cdc42p activity in the filamentous growth pathway based on genetic suppression analysis and in vitro kinase assays. Bem4p also interacted with the MAPKKK Ste11p *in vivo* and *in vitro* and the filamentous growth MAPK Kss1p *in vivo*, but not with the mating pathway MAPK, Fus3p, or HOG pathway MAPK, Hog1p. Accordingly, Bem4 was required to activate the filamentous growth pathway but not other MAPK pathways that share common components. Taken together, we have identified a new mechanism by which Cdc42 becomes activated and directed into a particular MAPK pathway by a Cdc42-interacting adaptor protein.

162C. Med13p anchors cyclin C in the nucleus to prevent stress-independent mitochondrial fragmentation and stress hypersensitivity. ***Katrina F. Cooper****, Svetlana Khakhina, Chunyan Jin.* Dept Molec Biol, Rowan University-SOM, Stratford, NJ.
The budding yeast cyclin C has two roles in the cell. First, in combination with Med12p, Med13p and Cdk8p, cyclin C forms a subcomplex (Cdk8 module) of the mediator that represses transcription initiation of several gene types including stress responsive loci. In its second role, cyclin C translocates to the cytoplasm in response to oxidative stress. In the cytoplasm, cyclin C associates with the mitochondria to promote extensive fission and programmed cell death. Therefore,

releasing cyclin C from the nucleus represents a critical decision point in the cellular response to stress. We demonstrate that Med13p represents the nuclear anchor that retains cyclin C in the nucleus in unstressed cells. Deleting MED13 allows cyclin C relocalization to the cytoplasm in the absence of stress. This event has three important consequences for the cell. First, the mitochondria undergo constitutive extensive fission. Second, since this fragmentation prevents mitochondrial DNA repair, large deletions occur in the mitochondrial genome resulting in loss of organelle function. Finally, mitochondrial hyper-fission hypersensitizes cells to programmed cell death. Further studies revealed two independent mechanisms that dissolve the Med13p-cyclin C interaction. First, cyclin C is phosphorylated by the Slt2p MAP kinase. This modification causes partial release of cyclin C from Med13p. However, final dissolution of the interaction requires Med13p proteolysis. Med13p destruction requires Cdk8p kinase activity and the 26S proteasome. Taken together, this study defines a complex molecular switch controlling cyclin C subcellular localization and ultimately mitochondrial shape, function and sensitivity to cytotoxic agents. This study reveals a new role for Med13p in maintaining mitochondrial maintenance and preventing aberrant activation of the programmed cell death pathway by restricting cyclin C to the nucleus in non-stressed cells.

163A. Tor1 and PKA downregulation in stationary phase rely on Mtl1 to preserve mitochondrial integrity and cell survival. *Venkatraghavan Sundaran[1], Mima Petkova[1], Nuria Pujol-Carrion[1], Jordi Boada[2],* ***Maria Angeles de la Torre-Ruiz***[1]. 1) Basic Medical Sciences-IRBLleida, University of Lleida, LLEIDA, Spain; 2) Experimental Biology-IRBLleida, University of Lleida, Lleida, Spain.
Budding yeast are a good model system to study aging in non dividing cells. Here we show that Mtl1, member of the Cell Wall Integrity pathway (CWI), plays a positive role in Chronological Life Span (CLS). The absence of Mtl1 shortens CLS and causes impairment in the mitochondrial function. This is reflected in a descent in oxygen consumption during the postdiauxic state, an increase in the uncoupled respiration and mitochondrial membrane potential and also a descent in aconitase activity. We demonstrate that all these effects are a consequence of signalling defects suppressed by *TOR1* deletion and less efficiently by PKA inactivation. The stability of the inhibitory subunit of PKA, Bcy1 is severely reduced in the absence of Mtl1, mainly as a consequence of a high PKA activity. Mtl1 also regulates PKA inactivation through Bcy1 phosphorylation, mainly in response to glucose depletion. In postdiauxic phase and in conditions of glucose depletion, Mtl1 negatively regulates *TOR1* function to phosphorylate Bcy1 and thus to inhibit PKA, although additional targets are not completely ruled out. Mtl1 links mitochondrial dysfunction with TOR and PKA pathways in quiescence, being glucose the main signalling molecule.

164B. Calcineurin regulates the yeast synaptojanin Inp53/Sjl3 during membrane stress. ***Evan Guiney***[1], *Joshua Elias*[2], *Martha Cyert*[1]. 1) Biology, Stanford University, Stanford, CA; 2) Chemical and Systems Biology, Stanford University, Stanford, CA.
The budding yeast *Saccharomyces cerevisiae* undergoes dramatic volume loss during hyperosmotic stress, leading to massive membrane invaginations. The cellular response to this membrane stress involves a large scale reorganization of the endocytic machinery, with actin patches, the sites of clathrin mediated endocytosis, undergoing a temporary depolarization. Neither the functional significance nor the molecular causes of this endocytic reorganization are well understood.
Here we show that the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin is activated during hyperosmotic stress. We find that hyperosmotic stress causes rapid changes in calcineurin localization, with a pronounced accumulation of calcineurin at sites of polarized growth. The role of calcineurin in hyperosmotic stress was previously unclear, because although high osmolarity causes elevated cytoplasmic Ca^{2+}, cells lacking calcineurin have no long term defects in growth rate. Instead, we find that calcineurin is critical for surviving the initial shock: inhibiting calcineurin causes a 70% decrease in viability after 4 hour exposure to hyperosmotic shock. Furthermore, calcineurin inhibition also exacerbated depolarization of the actin cytoskeleton, another rapid response to hyperosmotic shock.
We characterized the molecular mechanisms through which calcineurin acts during this acute response, and found calcineurin to bind to and dephosphorylate the yeast synaptojanin Inp53/Sjl3 during hypertonic shock. Cells expressing an Inp53 allele that cannot be regulated by calcineurin show depolarized actin and have numerous large plasma membrane invaginations, demonstrating that calcineurin promotes survival during hyperosmotic shock at least in part by activating Inp53. We hypothesize that calcineurin modulates Inp53 interactions with protein partners, and show that calcineurin-mediated dephosphorylation, and hypertonic shock-induced phosphorylation of Inp53 reduce Inp53's interaction with clathrin, and increase its interaction with Sla1, Bzz1, Bsp1, and Rvs167, all of which localize to actin patches.
Extensive similarities between this pathway and calcineurin-regulated synaptic vesicle endocytosis in neurons suggest that the activation of Ca^{2+}/CN signaling in response to excess membrane and subsequent formation of endocytic complexes is a fundamental and conserved regulatory feature of eukaryotic cells.

165C. TOR Complex 1 is a direct target of amino acid sensor Gcn2. *Wenjie Yuan,* ***Yu Jiang***. Department of Pharmacology and Chemical Biology, Univ Pittsburgh, Pittsburgh, PA.

In eukaryotic cells two conserved protein kinases Gcn2 and TOR complex 1 (TORC1) couple amino acid conditions to protein translation. Gcn2 is a well-established amino acid sensor and is activated by uncharged tRNAs which accumulate when amino acids are limited. Activated Gcn2 phosphorylates and inhibits eukaryotic initiation factor-2 (eIF2), resulting in repression of general protein synthesis. Like Gcn2, TORC1 is also involved in sensing amino acid conditions for controlling protein translation. However, the underlying mechanism is poorly defined. In the present study, we show that TORC1 is a direct target of Gcn2 kinase. In response to amino acid starvation, Gcn2 binds to TORC1 and phosphorylates Kog1, the unique regulatory subunit of TORC1, resulting in downregulation of TORC1 kinase activity. In the absence of Gcn2, TORC1 activity increases and becomes unresponsive to amino acid starvation. Our findings demonstrate that TORC1 is an effector of Gcn2 in amino acid signaling, hence defining a novel mechanism by which TORC1 senses amino acid conditions.

166A. Protein-Protein Interactions of the Yak1 Kinase. *Adeline Boettcher, Scott Blaszak, Samantha J. DeWerff,* ***Stephen D. Johnston***. Department of Biology, North Central College, Naperville, IL.
Yak1 is a dual-specificity kinase that has been implicated in diverse cellular responses to environmental stress. To better understand how the Yak1 kinase mediates these responses, we carried out a two-hybrid screen to identify proteins that physically interact with the noncatalytic amino-terminal domain. We discovered that the transcription factor Mss11p and the P-body protein Pat1p bind the amino terminal domain and have confirmed that Ypl247p binds this domain as well. The association between the amino-terminal domain of Yak1p and these three proteins is independent of the *RAS*/cAMP pathway or heat stress. Curiously, we find that these three proteins bind poorly to full-length Yak1p in a standard two-hybrid assay. However, if the *RAS*/cAMPpathway is deactivated by the overexpression of a phosphodiesterase, then Pat1p interacts with full-length Yak1p. Furthermore, the Bmh1 protein binds efficiently to full-length Yak1p, but this association is lost if the cells are subjected to heat stress. We have initiated a series of experiments to understand the physiological importance of these physical interactions. Cells lacking functional *YAK1* are sensitive to low concentrations of acetic acid, but only if the *PAT1* gene is intact. *pat1* cells show an increased rate of plasmid loss, which seems to be exacerbated by *YAK1* overexpression. In contrast, the overexpression of *MSS11* leads to haploid invasive growth, but only if the *YAK1* gene is intact. Deletion of either *YPL247c* or *YAK1* causes cells to accumulate unusually high concentrations of glycogen. Phd1p, but not Ino2p, Sfl1p or Tec1p, seems to be the key transcription factor governing Yak1p expression. Finally, we found a naturally existing stop codon in the *YAK1* homolog in *Saccharomyces bayanus* that truncates this protein. The amino-terminal domain is still expressed in *S. bayanus*, but its deletion leads to no measurable phenotype, suggesting that *YAK1* is an expressed pseudogene in this species.

167B. Endolysosomal membrane trafficking complexes drive nutrient-dependent TORC1 signaling to control cell growth in *Saccharomyces cerevisiae*. ***Joanne M Kingsbury***, *Neelam D Sen, Maria E Cardenas.* Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.
The Target of rapamycin complexes (TORC) are conserved across eukaryotes and orchestrate myriad cellular processes to control growth in response to nutrients and environmental signals. TORC1 is physically associated with endomembranes and controls cell growth when nutrients such as amino acids are abundant and serves to maintain robust nutrient transport, ribosome biogenesis, and protein synthesis, and concomitantly inhibits autophagy. We have shown that mutations in Class C Vps (Vps-C) complexes are synthetically lethal with *tor1* mutations and confer rapamycin hypersensitivity in *Saccharomyces cerevisiae*, suggesting a role for these complexes in TORC1 signaling. Vps-C complexes are required for vesicular trafficking and fusion, and comprise four distinct complexes; HOPS and CORVET and their minor intermediaries i-CORVET and i-HOPS. We show that at least one Vps-C complex is required to promote TORC1 activity, with the HOPS complex having the greatest input. The *vps-c* mutants fail to recover from rapamycin-induced growth arrest and show low levels of TORC1 activity. TORC1 promotes cell growth via Sch9, a $p70^{S6}$ kinase ortholog. Constitutively active *SCH9* or hyperactive *TOR1* alleles restored rapamycin recovery and TORC1 activity of *vps-c* mutants, supporting a role for the Vps-C complexes upstream of TORC1. The EGO GTPase complex (EGOC) and its homologous Rag-GTPase complex convey amino acid signals to TORC1 in yeast and mammals, respectively. Expression of activated EGOC GTPase subunits, $Gtr1^{GTP}$ and $Gtr2^{GDP}$, partially suppressed *vps-c* mutant rapamycin recovery defects, and this suppression was enhanced by increased amino acid concentrations. Moreover, *vps-c* mutations disrupted EGOC-TORC1 interactions. TORC1 defects were more severe for *vps-c* mutants than those observed in EGOC mutants. Taken together, our results support a model in which distinct endolysosomal trafficking Vps-C complexes promote rapamycin-sensitive TORC1 activity via multiple inputs, one of which involves maintenance of amino acid homeostasis that is sensed and transmitted to TORC1 via interactions with EGOC.

168C. Membrane fluidity and temperature sensing are coupled via circuitry comprised of Ole1, Rps5, and Hsf1 in *Candida albicans*. ***Michelle Leach***[1,2], *Leah Cowen*[1]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Aberdeen Fungal Group, University of Aberdeen, Institute of Medical Sciences, Aberdeen, UK.

Temperature is a ubiquitous environmental variable, which can profoundly influence the physiology of living cells as it changes over time and space. Coordination of sensing and responding to temperature shifts is crucial for organismal survival, and depends upon sophisticated mechanisms that have been uniquely tuned over evolutionary time. For pathogens, the ability to grow at 37C is essential for virulence in a mammalian host, with further increases in temperature in the form of fever being a prevalent host response to pathogen invasion. Cellular membranes are poised to serve as front line thermosensors, with changes in membrane fluidity acting as a rapid response to temperature fluctuations. Here, we explored the molecular mechanisms by which changes in membrane fluidity influence temperature sensing in the most prevalent human fungal pathogen, *Candida albicans*. We characterised the role of the fatty acid desaturase Ole1, which is responsible for synthesis of the unsaturated fatty acid oleic acid, during normal growth, high temperature growth and acute heat shocks. We determined that in response to elevated temperature, levels of *OLE1* increase. Subsequently, loss of Ole1 triggers expression of the fatty acid synthase *FAS2*. Furthermore, depletion of Ole1 prevents full activation of the heat shock transcription factor, Hsf1, thereby reducing heat shock protein gene expression in response to heat shock. This reduction in Hsf1 activation is attributable to the E3 ubiquitin ligase Rsp5, which regulates *OLE1* expression. Finally, misregulation of *OLE1* during increased growth temperatures leads to a fungicidal effect when cells are treated with the antifungal drug amphotericin B, which targets the cell membrane. Thus, this work illuminates circuitry linking membrane fluidity and the heat shock response, with broad implications for fungal pathogenesis.

169A. Regulation of the essential protein kinase Ypk1 by the TORC2 complex. ***Kristin Leskoske***, *Françoise Roelants, Jeremy Thorner*. Div. of Biochemistry, Biophysics and Structural Biology, Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720-3202 USA.
A eukaryotic plasma membrane exhibits an asymmetric distribution of lipids both within and between its leaflets. Lipid composition influences membrane properties, such as its curvature and fluidity, as well as the organization of signaling and structural proteins embedded in and associated with the membrane. Thus, it is important that cells are able to maintain proper lipid organization during the constant membrane remodeling that occurs during cell growth due to vesicle-mediated processes, such as exo- and endocytosis. In *Saccharomyces cerevisiae*, the 680-residue, AGC family protein kinase Ypk1 (along with its paralog Ypk2) is an important regulator of lipid membrane homeostasis. Ypk1-deficient mutants display defects in receptor internalization and endocytosis, as well as increased sensitivity to hyperosmotic stress and compounds that perturb sphingolipid synthesis, such as myriocin. Ypk1 regulates plasma membrane homeostasis in multiple ways, including stimulating sphingolipid synthesis, inhibiting aminophospholipid flipping, and modulating glycerol-3-phosphate production for glycerolipid synthesis. Ypk1 activity requires phosphorylation of T504 in the activation loop of its kinase domain (residues 342-602) by eisosome-associated Pkh1 (along with its paralog Pkh2), which are the yeast homologs of mammalian 3-phosphoinositide-dependent protein kinase-1 (PDK1). Full Ypk1 activity also requires its phosphorylation at C-terminal sites, dubbed its "turn" (S644) and "hydrophobic" (T662) motifs by the Target of Rapamycin Complex 2 (TORC2). We have now identified biochemically several additional C-terminal sites that are also phosphorylated in a TORC2-dependent manner and that, together, define a previously unrecognized consensus motif. Furthermore, we used genetic analysis to demonstrate that these newly identified sites are nearly as important for Ypk1 activity and biological function as the previously identified S644 and T662 sites. Ala substitution mutations at these sites abrogated the ability of the mutant protein to rescue the phenotypes of Ypk1 deficiency, whereas Glu substitution mutations at the same sites had no detectably deleterious affect. Furthermore, combining the Ala substitution mutations with an N-terminal point mutation (D242A), which, we have demonstrated before constitutively activates Ypk1, restored the ability of the resulting mutant protein to complement a Ypk1-deficient cell. These findings provide new insights about the molecular basis for TORC2-mediated activation of Ypk1.

170B. Latency of transcription factor Stp1 depends on a modular regulatory motif that functions as cytoplasmic retention determinant and nuclear degron. *Deike J. Omnus,* ***Per O. Ljungdahl***. Dept Molecular Biosciences, Wenner-Gren Inst, Stockholm Univ, Stockholm, Sweden.
The SPS sensing pathway enables yeast cells to respond to extracellular amino acids. In the absence of inducing amino acids the effector transcription factor of the pathway, Stp1, is maintained latent by cytoplasmic retention and promoter exclusion. The molecular basis underlying these distinct regulatory activities are poorly understood, but require the N-terminal domain of Stp1 and three inner nuclear membrane proteins, Asi1, Asi2 and Asi3. Here we have investigated Stp1 latency in the absence of signaling and report that the Stp1 N-terminal regulatory domain contains a small motif, designated RI, that fully accounts for the latent properties of Stp1. RI is modular; RI retains histone Htb2 in the cytoplasm as well as targets hSOS to the plasma membrane. Intriguingly, mutations in RI that diminish cytoplasmic retention exhibit enhanced Asi protein-dependent degradation; loss of function mutations in *ASI1*, *ASI2* or *ASI3* stabilize nuclear localized Stp1. These findings provide novel mechanistic insights into the SPS sensing pathway and demonstrate for the first time that the inner nuclear membrane Asi-proteins function in a degradation pathway in the nucleus.

171C. The phosphorylation state of *Saccharomyces cerevisiae* signaling proteins varies in a stress-dependent fashion. ***Matthew MacGilvray***[1], *Anna Larson*[2], *David Berry*[3], *Josh Coon*[2], *Audrey Gasch*[1]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison , WI; 2) Department of Chemistry, University of Wisconsin-Madison, Madison, WI; 3) Institute for Neurodegenerative Disease, UCSF School of Medicine, San Francisco, CA.
Saccharomyces cerevisiae adapts to stressful conditions by altering the expression of condition-specific genes as well as a common stress-activated expression program termed the environmental stress response (ESR). While gene expression alterations and their role in acclimation to many external stresses are characterized, knowledge of both the upstream regulatory network and the post-translational protein modifications that control these expression changes remains incomplete. The identities of many of the regulators involved, including kinases and phosphatases, transcription factors, and RNA binding proteins, is incomplete; furthermore, to what extent regulators are activated by a single stress versus many stresses is unknown. Toward the goal of identifying and comparing stress-activated signaling networks, our lab has developed a computational and experimental pipeline that accurately predicted the NaCl regulatory network in *S. cerevisiae*. This pipeline integrates gene fitness contributions, RNA-seq and phospho-proteomic data, and high-throughput protein interaction data sets to generate an inferred stress network. Here, we identified the post-translational component of the dithiothreitol (DTT) and heat shock (HS) signaling networks by performing quantitative phospho-proteomic analyses on the strain BY4741, subjected to either stress. We compared and contrasted phospho-peptides and their abundances across the three stresses examined, revealing shared and condition-specific phosphorylation changes in regulatory proteins. Taken together, these results are contributing to our understanding of condition-specific versus common regulators activated by stress.

172A. Substrate sequence specificity and catalytic properties of the signaling Ssy5 endoprotease. ***Antonio Martins***, *Per Ljungdahl.* Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.
The signaling Ssy5 endoprotease is one of three core components of the plasma membrane localized SPS-sensor. The SPS-sensor enables yeast cells to sense and respond to extracellular amino acids by upregulating their uptake. Ssy5 exhibits homology to chymotrypsin-like serine proteases and is expressed as a zymogen. During biogenesis, Ssy5 cleaves itself into an N-terminal pro- and a C-terminal catalytic (Cat)-domain; the pro- and Cat-domains remain non-covalently associated, and the prodomain inhibits the proteolytic activity of the Cat-domain. Upon induction, the SPS-sensor orchestrates the proteasomal degradation of the prodomain, the unfettered Cat-domain cleaves the effector transcription factors Stp1/2, and the processed factors target the nucleus and induce transcription of amino acid permease genes. Little is known regarding the catalytic properties of Ssy5. Broad-spectrum protease inhibitor cocktails and specific serine protease inhibitors fail to inhibit processing, indicating that access to the active site is restricted. To understand Ssy5 function, we have identified the scissile bonds (P1-P'1) in both Stp1 and Stp2. This was accomplished using a cysteine scanning approach coupled with maleimide-PEG treatment to obtain diagnostic mobility shifts observable by SDS-PAGE. The results show that Ssy5 cleaves between a cysteine (P1) and serine or alanine (P'1). Site-specific mutagenesis, targeting residues comprising the cleavage sites of Stp1/Stp2, confirmed the requirement for small residues (cysteine, serine or alanine) at the P1 position. Cleavage of Stp1, but not Stp2, was impaired when charged residues (lysine or aspartate) were placed at the P4 position. The catalytic properties of Ssy5 are being further probed by site-specific replacements of conserved residues located in the active site cleft, which in other well-characterized serine proteases are known to affect substrate specificity. Mutations that render Ssy5 sensitive to specific serine proteases inhibitors are being sought. The determination of the cleavage site in Stp1 and Stp2, and defining of Ssy5 sequence specificity determinants are providing new insights regarding the catalytic properties of Ssy5.

173B. TORC1 regulates the yeast lipin Pah1 via the Nem1/Spo7 protein phosphatase complex. *Emmanuelle Dubots, Stéphanie Cottier, Marie-Pierre Péli-Gulli, Malika Jaquenoud, Séverine Bontron,* ***Marta Moreno Torres****, Roger Schneiter, Claudio De Virgilio.* Department of Biology, Unit of Biochemistry, University of Fribourg, Fribourg, Switzerland.
Storage and degradation of triacylglycerol (TAG) are nutrient-regulated processes that play important roles in homeostasis of cellular energy and membrane biosynthesis. The signaling cascades that regulate TAG homeostasis in response to nutrient cues, however, remain poorly understood. Here we show that the evolutionary conserved target of rapamycin complex 1 (TORC1) prevents the accumulation of TAG by (indirectly) inhibiting the phosphatidate (PA) phosphatase Pah1 that generates diacylglycerol (DAG) from PA. Using metabolic lipid-radiolabeling assays, we found that inhibition of TORC1 with rapamycin leads to a net increase in TAG synthesis in vivo in a Pah1-dependent manner. Rapamycin-induced TAG synthesis further required the acyl-CoA:diacylglycerolacyltransferase Dga1, which forms TAG from acyl-CoA and DAG. Pah1 is a highly regulated phosphoprotein that is activated by dephosphorylation at the ER membrane via the heterodimeric Nem1-Spo7 protein phosphatase complex. We observed that TORC1 inhibition resulted, in parallel to a modest increase of the interaction between Pah1 and the Nem1-Spo7 module, in specific phosphorylation of Nem1, which is required for proper regulation of Pah1. Our results delineate a new TORC1 effector branch that controls lipin function in yeast, which may, based on the recent discovery of Nem1-Spo7 orthologous proteins in humans, potentially be conserved.

174C. TORC2-dependent Protein Kinase Ypk1 Phosphorylates Ceramide Synthase Components Lag1 and Lac1 to Stimulate Sphingolipid Synthesis. ***Alexander Muir***, *Subramaniam Ramachandran, Françoise Roelants, Garrett Timmons, Jeremy Thorner.* Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.
The plasma membrane of a eukaryotic cell is a highly organized structure with a unique lipid and protein composition. Maintenance of the content and arrangement of plasma membrane lipids is essential for cell survival and needs to be preserved during normal cellular processes and in the face of environmental insults that perturb membrane organization. How plasma membrane lipid composition is perceived, and the mechanisms for adjusting the abundance and distribution of the lipids, are largely unknown. Recently, we showed that the TOR Complex 2 (TORC2)-Ypk1 signaling axis is an important regulatory module for controlling sphingolipid metabolism through phosphorylation of Orm1 and Orm2, which relieves their inhibition of the serine:palmitoyl-CoA acyltransferase complex, especially in response to membrane stressors. To explore whether TORC2-Ypk1 signaling has additional roles in homeostatic control of lipid biogenesis, we performed an unbiased multi-step procedure to identify Ypk1 substrates by applying: (a) bioinformatic analysis to catalog potential candidates; (b) a novel modification of the synthetic dosage lethality screen to narrow down the list; (c) confirmation that the resulting gene products are substrates for Ypk1 in vitro; and, (d) corroboration that such substrates are phosphorylated in vivo in a Ypk1-dependent manner and at its Ypk1 phosphoacceptor sites. In this way, we identified novel Ypk1 target involved in membrane homeostasis, including both Lac1 and Lag1, catalytic components of the ceramide synthase complex. Phosphorylation of Lac1 and Lag1 increases in response to sphingolipid depletion and phosphorylation is important for optimal cell survival under such conditions. Calcineurin/phosphoprotein phosphatase 2B contributes to the dephosphorylation of these proteins. Mass spectrometry and metabolic labeling studies indicate that phosphomimetic mutations, Lac(S23E S24E), increase flux through the sphingolipid pathway, whereas an unphosphorylatable mutant, Lac(S23A S24A), causes accumulation of long chain bases, as expected if phosphorylation enhances ceramide synthase function. In vitro experiments confirm that Ypk1 phosphorylation directly enhances ceramide synthase activity. Hence, Lac1 and Lag1 are physiologically relevant Ypk1 substrates. Thus, TORC2-Ypk1 signaling coordinately regulates sphingolipid metabolism both at the level of long chain base production and at the level of ceramide synthesis, thereby allowing the cell to adjust to membrane perturbation.

175A. Target of rapamycin-responsiveness on the GATA-family transcription activator Gln3. ***Rajendra Rai***[1], *Jennifer J. Tate*[1], *Karthik Shanmuganatham*[2], *Martha M. Howe*[1], *Terrance G. Cooper*[1]. 1) Dept. Microbiol., Immunol. and Biochem., Univ. Tennessee, Memphis, TN; 2) Dept. Infectious Disease, St. Jude Children's Research Hospital, Memphis, TN.
Nitrogen-responsive control of Gln3 localization is implemented through distinct TorC1-dependent (rapamycin-responsive) and TorC1-independent (nitrogen catabolite repression-sensitive and methionine sulfoximine- [Msx-] responsive) regulatory pathways. We previously demonstrated that a 17 amino acid peptide containing a sequence with a propensity to fold into an -helix, $Gln3_{656-666}$, was necessary and sufficient for a strong, two-hybrid Gln3-Tor1 interaction. One side of this putative helix was very hydrophobic and flanked by serine residues. Substitution of aspartate or alanine for these serines eliminated the Gln3-Tor1 interaction, rapamycin-responsiveness of Gln3 localization and partially abrogated cytoplasmic Gln3 sequestration in cells cultured under nitrogen repressive conditions. Here we demonstrate those three characteristics are not inextricably linked together. We identified a second distinct Gln3 region ($Gln3_{510-589}$) that is specifically required for the rapamycin-responsiveness of Gln3 localization, but is not required for cytoplasmic Gln3 sequestration under repressive growth conditions or for Gln3 relocation to the nucleus following Msx addition. Aspartate substitutions for residues throughout this region uniformly abolish rapamycin-responsiveness. Contained within this region is a sequence that also possesses a predicted propensity to form an $-helix_{583-591}$. One side of this putative helix consists of three hydrophobic amino acids flanked by serine residues. Substitution of aspartate for even one of these serines abolishes the Gln3 response to rapamycin and increases rapamycin resistance without affecting either of the other two Gln3 localization responses. Further, they have no effect on the Gln3-Tor1 interaction. In contrast, alanine substitutions decrease rapamycin resistance. Together, these data suggest that targets in the C-terminal portion of Gln3 required for the Gln3-Tor1 interaction, cytoplasmic Gln3 sequestration, and Gln3 responsiveness to Msx addition and growth in poor nitrogen sources are distinct from those needed for rapamycin-responsiveness. Supported by NIH grant GM-35642.

176B. Endocytosis and vacuolar degradation of the yeast cell surface glucose sensors Rgt2 and Snf3. ***A. Roy***, *J.H Kim.* Biochemistry and Molecular Medicine, The George Washington University, Washington , DC.
Sensing and signaling the presence of extracellular glucose is crucial for the yeast Saccharomyces cerevisiae because of its fermentative metabolism, characterized by high glucose flux through glycolysis. The yeast senses glucose through the cell surface glucose sensors Rgt2 and Snf3, which serve as glucose receptors that generate the signal for induction of genes involved in glucose uptake and metabolism. Rgt2 and Snf3 detect high and low glucose concentrations, respectively, perhaps due to their different affinities for glucose. Here, we provide evidence that cell surface levels of glucose sensors are regulated by ubiquitination and degradation in the vacuole. The glucose sensors are removed from the plasma membrane through endocytosis and targeted to the vacuole for degradation upon glucose depletion. The turnover of the

glucose sensor is inhibited in endocytosis defective mutants, and the sensor proteins with a mutation at their putative ubiquitin-acceptor lysine residues are resistant to degradation. Of note, the low affinity glucose sensor Rgt2 remains stable only in high-glucose grown cells, and the high affinity glucose sensor Snf3, only in cells grown in low glucose. Besides, constitutively active, signaling forms of glucose sensors do not undergo endocytosis, whereas signaling defective sensors are constitutively targeted for degradation, suggesting that the stability of the glucose sensors may be associated with their ability to sense glucose and that thus the amount of glucose available dictates the cell surface levels of the glucose sensors. Therefore, differential regulation of the cell surface levels of glucose sensors may enable yeast cells to maintain glucose sensing activity at the cell surface over a wide range of glucose concentrations and to respond rapidly to changing glucose levels.

177C. Functional characterization of protein interactors of Wsc1p and Mid2p stress sensors and *PKC1* signaling in *Saccharomyces cerevisiae*. ***Ednalise Santiago-Cartagena***[1], *Vladimir Vélez-Segarra*[1], *Igor Stagljar*[2], *Brian C. Rymond*[3], *José R. Rodriguez-Medina*[1]. 1) Biochemistry, University of Puerto Rico- Medical Sciences Campus, San Juan, PR; 2) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Canada; 3) University of Kentucky, Lexington, KY.
The fungal cell wall is necessary to maintain cell morphology and cell integrity under environmental stress conditions. Signaling proteins required for activating the yeast *PKC1* pathway are an attractive target for antifungal drugs because they contribute to cell viability under cell wall stress. The objective of this research is to identify proteins that interact with the main sensors of the *PKC1* pathway and determine if these factors contribute to *PKC1* activation. We also want to determine the susceptibility of strains bearing null mutations for genes encoding stress sensor proteins and their interacting protein partners to antifungal drugs or environmental stress. Our hypothesis is that interacting partners of the Wsc1p and Mid2p stress sensors are required for resistance to antifungal drugs or environmental stress. To identify novel interacting proteins, the integrated Membrane Yeast Two-Hybrid (iMYTH) technique was applied. Strains bearing null mutations for genes encoding stress sensor proteins and their interacting proteins were tested for growth on medium containing the stressors Calcofluor White (CW), H_2O_2, Caspofungin (CS), Amphotericin B (AmpB), or under exposure to thermal stress at 37C. In addition, these mutant strains were tested for their ability to activate the *PKC1* pathway. Results showed that at 30C, 14 novel interactors were confirmed for Wsc1p and 29 for Mid2p. 15 of the null mutant strains acquired sensitivity to AmpB (75g/ml). 9 of the null mutants acquired resistance to 1mM H_2O_2, while two acquired sensitivity to H_2O_2 and were impaired in *PKC1* signaling. 4 of the null mutant strains acquired sensitivity to CS (75ng/ml) while 6 acquired resistance to CS. The wsc1 null mutant was the only strain that showed sensitivity to CW (150g/mL). In conclusion, signaling proteins involved in *PKC1* pathway regulation were shown to be associated with survival under different stresses. This research was supported by the UPR School of Medicine, Univ. of Toronto, Univ. of Kentucky, NIGMS/NIAID-SC1AI081658, NCRR-RCMI-G12MD007600 and MBRS-RISE-R25GM061838.

178A. Plasma membrane flippase function is required for signaling competence in the mating pheromone response pathway. ***Elodie A. Sartorel***, *Evelyne Barrey, Rebecca Lau, Jeremy Thorner.* Molecular and cell biology, University California Berkeley, Berkeley, CA.
In the plasma membrane (PM) of eukaryotic cells, phospholipid asymmetry appears to be important for cell signaling and cytokinesis. The outer leaflet of the PM contains predominantly phosphatidylcholine and sphingolipids, whereas the inner leaflet is enriched in phosphatidylserine (PtdSer), phosphatidylethanolamine (PtdEth) and phosphatidylinositol (PtdIns) and its phosphorylated derivatives (especially PI4P and PIP2). Members of the class 4 P-type ATPases ("flippases") maintain membrane lipid asymmetry by translocating PtdSer and PtdEth from the outer leaflet to the cytosolic leaflet of the PM. In *S. cerevisiae*, five genes (DNF1, DNF2, DNF3, DRS2 and NEO1) encode flippases. In *MAT***a** cells responding to -factor, we found that Dnf1, Dnf2 and Dnf3, and a flippase activator (the protein kinase Fpk1) localize at the shmoo tip where polarized growth is occurring and where Ste5 (the central scaffold protein of the pheromone MAPK cascade) is recruited. Hence, we used genetic analysis to explore whether flippase function has any role in pheromone signaling. We found that a *MAT***a** *dnf1 dnf2 dnf3* triple mutant exhibits a marked reduction in its ability to respond to -factor and determined that, in these cells, there is a pronounced reduction in the stability of Ste5 resulting from an elevated rate of its Cln2Cdc28-dependent degradation. Similarly, we found that a *MAT***a** *dnf1 dnf3 drs2* mutant also displays a reduction in its ability to respond to -factor; however, in these cells, the defect was attributable to inefficient recruitment of Ste5 to the PM because of severe mislocalization of the PI4P and PIP2 pools. In addition, biochemical analysis using phosphorylation-dependent electrophoretic mobility shifts in a C-terminal segment of Dnf1 as a measure of Fpk1 and Fus3 (and Kss1) activity in vivo demonstrates an increase in flippase phosphorylation when cells are exposed to mating pheromone. These observations indicate that maintenance of glycerolipid asymmetry is necessary for efficient PM recruitment and function of the pheromone signaling apparatus.

179B. Role of the Glc7-Reg1/2 phosphatase in the control of the Mig1 transcriptional repressor. ***Sviatlana Shashkova***[1], *Raul Garcia-Salcedo*[1], *Loubna Bendrioua*[1], *Timo Lubitz*[2], *Niek Welkenhuysen*[1], *Edda Klipp*[2], *Stefan Hohmann*[1]. 1)

Chemistry and Molecular Biology, University of Gothenburg, Sweden; 2) Theoretical Biophysics, Humboldt-Universität zu Berlin, Germany.
The yeast AMPK/SNF1 signalling pathway controls energy homeostasis and is best known for its role in glucose derepression. When glucose becomes limiting, three upstream kinases phosphorylate and activate Snf1, which in turns phosphorylates and inactivates the Mig1 transcriptional repressor to lift glucose repression. Upon glucose repletion, the Glc7-Reg1 phosphatase dephosphorylates and inactivates Snf1. Also Mig1 becomes rapidly dephosphorylated following glucose re-addition, accumulates in the nucleus and mediates repression of a large number of genes. Mig1 dephosphorylation is reported in the literature to be mediated by the same protein phosphatase (Glc7-Reg1) that also dephosphorylates Snf1. However, there is no direct evidence supporting this idea: despite the fact that protein interactions in the Snf1-Mig1 system have been extensively studied, no interaction linking Mig1 to Glc7-Reg1 has been reported. In addition, there is no apparent reason for employing the same phosphatase for the kinase and its target, in particular because glucose regulation of Snf1 dephosphorylation seems to be controlled mainly by the kinase itself. We re-investigated the possible role of Glc7-Reg1. Data-driven mathematical modelling and simulation indicates that a constitutive phosphatase acting on Mig1 may be sufficient to explain experimental time course data. Strong overexpression of Reg1 did not affect the Snf1 phosphorylation pattern but caused diminished expression of SUC2 as well as partially constitutive nuclear Mig1, consistent with Glc7-Reg1 action towards Mig1. At the same time it appears that while Snf1-phosphorylation is completely unregulated in the absence of Reg1/2, Mig1 still shows glucose-induced as well as Snf1 inhibitor-induced dephosphorylation. We also found that although Mig1 is cytosolic in the absence and nuclear in the presence of glucose it always shuttles between two compartments. In conclusion, it appears that Mig1 dephosphorylation may occur both inside and outside the nucleus and may be mediated by different phosphatases, where Glc7-Reg1 remains a candidate.

180C. Components of the Vid30 complex participates in the transcriptional regulation of glucose-repressed genes in *Saccharomyces cerevisiae*. *Angus Ross*, *Chris Snowdon*, *Andrew Fletcher*, ***George van der Merwe***. Department of Molecular & Cellular Biology, University of Guelph, Guelph, Canada.
Yeast has the ability to grow on a range of different carbon sources. In the presence of glucose (the preferred carbon source), the metabolism of non-fermentable carbon sources such as ethanol, acetate, and glycerol is inhibited by transcriptional repression and targeted protein inactivation and degradation. For example, the transcription of gluconeogenic and respiratory genes are repressed when abundant glucose is available. In addition, the gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase) is ubiquitylated and degraded when glucose-starved cells are replenished with glucose. This phenomenon, known as glucose repression or glucose regulation, is governed by a complex, tightly-regulated signaling network. Signaling pathways active in glucose abundant conditions, such as the Ras2/cAMP PKA pathway, participate in the transcriptional repression of glucose-repressed genes by inhibiting the function of the Snf1 kinase, which in turn is responsible for the derepression of these genes when glucose is absent. Snf1 is active in the absence of glucose and functions by inhibiting the transcriptional repressor proteins needed for the repression of glucose-repressed genes. Also, active Snf1 activates several transcriptional activators responsible for the derepression of glucose-repressed genes in the absence of glucose. The Vid30 complex (Vid30c)/GID complex is an E3 ubiquitin ligase known for its involvement in the degradation of FBPase, malate dehydrogenase, and the hexose transporter Hxt3 in different carbon conditions. Here we show the Vid30c also impacts carbon metabolism via gene transcription. Specifically, the Vid30c is required for the derepression of glucose-repressed genes when glucose is absent. We further show that the Vid30c may exert its influence on transcription by affecting the subcellular localization, protein concentration, and/or phosphorylation state of some glucose-regulated signaling molecules.

181A. Dynamics of MAPK signaling in *Saccharomyces cerevisiae*. ***Sarah Weisser***[1,3], *Konstanze Bandmann*[2,3], *Julia van der Felden*[1,3], *Peter Lenz*[2,3], *Hans-Ulrich Mösch*[1,3]. 1) Department of Genetics, Philipps-Universität Marburg, Karl-von-Frisch-Stra□e, 35032 Marburg, Germany; 2) Department of Physics, P□ 3, 35037 Marburg, Germany; 3) LOEWE-Center for Synthetic Microbiology (SYNMIKRO), Hans-Meerwein-Stra□e, 35043 Marburg.
In most eukaryotes, mitogen-activated protein kinase (MAPK) pathways have been found to control downstream processes by acting through specific transcription factors. In *Saccharomyces cerevisiae*, the Fus3/Kss1 MAPK module differentially controls several developmental programs including mating and multicellular development, for example biofilm formation and filamentation. For induction of the mating program, a pheromone signal is required that strongly stimulates the MAPK module and leads to activation of mating-specific genes by the transcription factor Ste12. In contrast, activation of multicellular growth programs and corresponding genes is achieved by stimulation of the MAPK module through a nutritional signal and by the transcription factor Tec1. Previous studies have revealed detailed insights into the wiring of many of the Fus3/Kss1 MAPK module components, for example into the complex interactions between the transcription factors Tec1 and Ste12. How the dynamic behavior of individual module components affects the correct processing of different input signals in single cells, however, is not well understood. In this study, we have established a fluorescence microscopy-based system for quantitative measurement of MAPK signaling *in vivo* at the single cell level with a focus on

Ste12 and Tec1 activity. In addition, we have started to develop mathematical models to describe the activity of the transcription factors in response to activation of the upstream MAP kinases. We will present evidence for the functionality of our *in vivo* system for MAPK module activity by showing that average data obtained by single cell measurements are comparable to previous biochemical measurements at the population level. We will also present our current efforts to determine significant cell to cell variation of MAPK activity and to analyze different regulatory sub-circuits within the MAPK module.

182B. Glucose derepression via Snf1-Mig1 is controlled at different levels. ***Niek Welkenhuysen***, *Tian Ye, Raul Garcia-Salcedo, Stefan Hohmann.* Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden.
The yeast AMPK/SNF1 signalling pathway controls energy homeostasis and is best known for its role in glucose de/repression. Upon limitation of preferred carbon sources, like glucose, Snf1 is phosphorylated and activated. Subsequently, the activated SNF1 complex phosphorylates and inactivates the Mig1 transcriptional repressor, thereby enabling expression of genes required for the utilisation of alternative carbon sources. Glucose derepression is an at the minimum two-step process: in the presence of glucose, active Snf1 is not sufficient to mediate glucose derepression. To further dissect the control of Snf1 and Mig1 phosphorylation we revisited the role of the two hexokinases, Hxk1 and Hxk2, and the glucokinase, Glk1, which together catalyse the first step of glycolysis. Hxk2 is required for glucose repression: an *hxk2* mutant constitutively expresses glucose repressed genes, such as SUC2 encoding invertase. It has long been a matter of debate if Hxk2 mediates this effect via its metabolic or a direct regulatory role. Here we show that all three sugar kinases contribute to the Snf1 phosphorylation state and only a mutant that lacks all three sugar kinases, *hxk1 hxk2 glk1*, displays complete absence of glucose-induced Snf1 dephosphorylation. This suggests that the role of the sugar kinases in controlling the Snf1 phosphorylation state is linked to metabolism. Consistently, strong overexpression of GLK1 in an *hxk1hxk2* mutant partly re-establishes glucose-induced Snf1 dephosphorylation but does not restore the function of the downstream elements of the Snf1-Mig1 system. Certain mutants defective in glucose metabolism downstream of sugar phosphorylation lack glucose-induced Snf1 dephosphorylation but still show glucose repression of invertase. Taken together, these data are consistent with a scenario where Hxk2 both has catalytic and regulatory roles in glucose de/repression: (1) together with Hxk1 and Glk1, Hxk2 controls the Snf1 phosphorylation state via its role in glycolysis, perhaps by affecting the ATP/ADP/AMP ratio; (2) in addition, Hxk2 may mediate glucose control of the access of active Snf1 to Mig1 in the nucleus, as suggested by data reported in the literature. Such a two-step system would allow for differential target selection: Snf1 may potentially be activated by any condition that affects the cellular energy balance while only glucose depletion would specifically mediate glucose derepression by Snf1-dependent Mig1 inactivation.

183C. Dissecting the role of calcineurin and protein kinase C signalling in Hsp90-dependent caspofungin tolerance. ***Jinglin L. Xie***[1], *Michelle D. Leach*[1,2], *Leah E. Cowen*[1]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Aberdeen Fungal Group, School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Abderdeen, United Kingdom.
Candida albicans is the major etiological agent of fungal infections in humans. Although antifungal drugs are routinely used to treat Candida infections, their therapeutic efficacy is limited by the basal drug tolerance of fungal pathogens, which can enable the evolution of drug resistance. The essential molecular chaperone heat shock protein 90 (Hsp90) is a key modulator of drug tolerance and resistance in C. albicans. Recent studies suggest that Hsp90 stimulates responses to drug-induced cell wall stress through its client protein calcineurin and the cell wall integrity (CWI) pathway mediated by protein kinase C (PKC). Here, we focus on elucidating the Hsp90-dependent regulatory circuitry that controls tolerance to echinocandins, which target the fungal cell wall and are the only new class of antifungal to reach the clinic in decades. Consistent with previous findings that deletion of PKC1 phenocopies inhibition of Hsp90 function, we found that Hsp90 regulates CWI signalling by stabilizing Pkc1. We confirmed that Pkc1 is an Hsp90 client in a co-immunoprecipitation experiment where Hsp90 co-purifies with Pkc1. Genetic epistasis analyses indicate that the recently identified transcription factor and regulator of echinocandin tolerance, Cas5, functions downstream of the Hsp90 clients Pkc1 and calcineurin. Quantitative RT-PCR analysis of transcript levels demonstrated that Cas5 regulates the expression of CWI gene PGA13, as well as calcineurin-dependent transcript ECM331 in response to caspofungin. We found that Cas5 protein stability is subjected to complex regulation by post-translational modifications under both basal and caspofungin treatment conditions. Pkc1 is required for the maintenance of Cas5 stability under basal conditions and calcineurin regulates Cas5 stability in response to drug-induced cell wall stress suggesting that Cas5 stability may be contingent upon dynamic changes in phosphorylation status. We are currently deciphering the Pkc1- and calcineurin-dependent post-translational modifications that regulate Cas5 stability by mass spectrometry and site directed mutagenesis. Our work illuminates novel cellular circuitry through which Hsp90 regulates drug tolerance and implicates Cas5, a fungal-specific protein downstream of Pkc1 and calcineurin, as a potential therapeutic target for the treatment of life-threatening fungal diseases.

184A. TORC1 and TORC2 regulate Rps6 phosphorylation via Ypk1/2/3 in budding yeast. ***Seda Yerlikaya***[1], *Madeleine Meusburger*[1], *Alexandre Huber*[1], *Dorothea Anrather*[2], *Gustav Ammerer*[2], *Robbie Loewith*[1,3]. 1) Molecular Biology, University of Geneva, Geneva, Switzerland; 2) Max F. Perutz Laboratories, Department of Biochemistry, University of Vienna, Dr. Bohrgasse 9, A1030 Vienna, Austria; 3) Swiss National Centre for Competence in Research Programme Chemical Biology, Geneva 1211, Switzerland.
For the past 30 years, the function of the nutrient-regulated phosphorylation of ribosomal protein S6 has remained mysterious. Probing this problem in the model eukaryote Saccharomyces cerevisiae we found that phosphorylation of the two phosphosites in yeast Rps6 are differentially regulated downstream of Target Of Rapamycin Complexes 1 and 2. TORC1 regulates phosphorylation of both sites directly via the poorly characterized AGC-family kinase Ypk3, and indirectly via Sch9 and its control on mRNA translation. TORC2 regulates phosphorylation of only the N-terminal phosphosite via Ypk1 and Ypk2. Glc7, the yeast PP1 phosphatase mediates Rps6 dephosphorylation. Strains harboring non-phosphorylatable variants of Rps6 have scorable phenotypes such as a significantly reduced growth rate. Currently, we are examining the role of Rps6 phosphorylation and the yeast S6 kinases in the genome-wide regulation of translation employing the ribosome profiling method. The results of this analysis will be presented.

185B. Mechanisms of nitrogen in regulating cAMP signal in Saccharomyces cerevisiae. *Y. Li*[1], ***A. Zhang***[1,2], *H. Jin*[1]. 1) Hebei University of Technology, No8 Guangrong Road,Hongqiao District, Tianjin,China; 2) Tianjin University, No92 Weijin Road, Nankai District, Tianjin, China.
Here we report mechanisms of nitrogen regulating cAMP signal in Saccharomyces cerevisiae. cAMP level were determined according to earlier report [1]. Addition of 100 mM glucose to glycerol-grown wild type cells caused a transient spike in cAMP level (Fig.1A). However, this glucose-induced cAMP signal disappeared in absence of nitrogen (Fig.1A). This indicates that nitrogen is required in the control of cAMP synthesis. Rapamycin treated cells have similar phenotype to nitrogen starvation cells. And TORC1 is targets of rapamycin. As shown in Fig.2, glucose-induced cAMP signal decreased significantly in rapamycin treated wild type cells compared with untreated cells. While rapamycin did not affect glucose-induced cAMP signal in rapamycin insensitive Tor1-RR cells (Fig.3), which contains the Ser1972-Ile mutant into wild type cells conferred dominant rapamycin resistance. As shown in Fig.4, Cdc25 was hyper-phosphorylated in rapamycin treated cells. Hyphosphorylated Cdc25 might cause lower activities of Ras proteins in rapamycin treated cells. So adenylae cyclase couldnt be activated effectively. These perhaps could explain why rapamycin treatment resulted in lower cAMP level (Fig.2). These indicate that nitrogen might regulate cAMP signal via TORC1. Effects of Sch9 on glucose-induced cAMP signal were also examined. There has a transient spike in cAMP level after addition of 100 mM glucose to glycerol-grown SCH9 deletion cells in the absence of nitrogen (Fig.4). This indicates that Sch9 might negatively regulate cAMP signal in the absence of nitrogen. Cdc25 have lower phosphorylation level in wild type cells and SCH9 deletion cells under nitrogen starvation, and there are no visible differences between wild type cells and SCH9 deletion cells. Deletion of SCH9 might increase cAMP synthesis directly under nitrogen starvation. Conclusion: Our researches demonstrate that nitrogen is required in cAMP synthesis. We also suggest that nitrogen regulate cAMP level via TORC1 and Sch9.

186C. Characterization of the Recruitment of Casein Kinase 1 to P Bodies. ***Bo Zhang***, *Khyati Shah, Qian Shi, Paul Herman.* Molecular Genetics, The Ohio State University, Columbus, OH 43210.
A variety of evolutionarily-conserved, cytoplasmic ribonucleoprotein (RNP) granules that contain specific mRNAs and RNA-binding proteins form in response to stress or particular developmental stimuli. Despite this conservation, the physiological functions associated with most of these structures have not yet been worked out. In some cases, such as with particular embryonic germ granules, it is known the mRNA present is being stored for later use. Recently, work from our lab and others has indicated that many key signaling proteins are also recruited to two of the best-characterized RNP granules in eukaryotic cells--processing bodies (P bodies) and stress granules. In all, these data suggest that the formation of RNP granules may provide an additional level of compartmentalization within the eukaryotic cytoplasm and that granule formation might influence a diverse set of biological processes. To test this possibility, we have been examining the Hrr25 protein kinase in order to identify the mechanisms for, and biological significance of, its recruitment to RNP granules. Hrr25 is the S. cerevisiae ortholog of the mammalian Casein Kinase I / enzyme (CK1/). This enzyme influences a number of essential cellular processes, including ribosome maturation, DNA repair and meiosis, in a manner that is conserved from yeast to humans. Interestingly, previous work has indicated that Hrr25 is a relatively promiscuous enzyme and that its activity is often controlled by directing the enzyme to appropriate locales in the cell; this localization can bring Hrr25 to regions that contain its physiologically-relevant substrates. In dividing cells, Hrr25 is found at the nucleus, spindle pole bodies and bud neck, depending upon the stage of the cell cycle. In this study, we found that Hrr25 re-localizes specifically to P bodies during periods of nutrient deprivation and other types of stress. This localization is reversible and Hrr25 foci rapidly disassemble upon removal of the inducing stress. Moreover, this association with P bodies is dependent upon Hrr25 kinase activity and particular protein constituents of these granules. Our current efforts

aim to more precisely define this localization pathway and to determine the physiological consequences of sequestering this enzyme within an RNP granule.

187A. An overexpression suppressor screen to identify genes that are effectors of nuclear morphology in Saccharomyces cerevisiae. ***James T. Arnone***, *Orna Cohen-Fix.* Laboratory of Cell and Molecular Biology, NIDDK/NIH, Bethesda, MD.
One of the defining characteristics of a eukaryotic cell is the nucleus, the membrane delimited region that houses the gDNA. Though the shape of the nucleus may vary by cell type, the maintenance of proper nuclear morphology is vital to cellular health. The loss of an appropriate nuclear morphology is a characteristic of certain diseases, including cancers, and occurs during the aging process. Unlike the canonical open-mitosis seen in many higher eukaryotes, the budding yeast, S. cerevisiae, undergoes closed mitosis - expanding the nuclear envelope throughout the cell-cycle and mitosis to allow for proper chromosome segregation to occur. Previous work from our lab and others has identified the Spo7-Nem1 phosphatase complex as necessary for normal nuclear morphology. This complex regulates Pah1p, the budding yeast lipin homologue, which in turn regulates the production of membrane phospholipids. The deletion of spo7 results in an accumulation of nuclear membrane adjacent to the nucleolus to form a nuclear envelope extension called a flare. We found that the spo7 strain is also sensitive to the translational inhibitor cycloheximide and has a reduced chronological and replicative life span. It is currently unclear how the absence of Spo7 leads to any of the spo7 strain phenotypes, nor is it known whether these phenotypes are dependent on each other. Nonetheless, we reasoned that identifying genes that, when over-expressed, rescue the cycloheximide sensitivity may lead to a better understanding of the nuclear and aging defects. Starting with a 2- based library containing genomic DNA inserts, we have identified 13 plasmids that rescued the cycloheximide sensitivity of the spo7 strain. Of those, 5 plasmids also suppressed the nuclear flare and 5 restored chronological life span to wild type levels. We are in the process of cloning and identifying the genes that are responsible for suppressing these phenotypes. So far we determined that AAC3, a gene coding for an adenine nucleotide translocator in the mitochondrial membrane, provides a partial rescue of the nuclear flare phenotype.

188B. A cell biological screen for age dependent changes in lipid metabolic pathways reveals changes in sphingolipid pathways. ***Anthony O Beas***, *Karen L Zhao, Daniel E Gottschling.* Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.
Age is the greatest risk factor for disease in the developed world, but many of the mechanisms that contribute to aging and disease remain unclear. In an effort to identify mechanisms that might contribute to the aging process, our lab has been using the budding yeast, Saccharomyces cerevisiae, as a model system to identify cellular changes that occur in mother cells during replicative aging. Using a combination of genetic and cell biology screens, we identified a number of age-dependent changes in yeast cells that are also observed in human cells. In the course of these studies, we examined whether age-dependent changes occur in the components of several conserved lipid metabolic pathways (e.g., fatty acids, neutral lipids, sphingolipids, glycerolipids). We specifically followed the distribution and levels of GFP tagged enzymes that synthesize or metabolize these lipids. Most enzymes remained the same during aging. However, we identified specific, age dependent changes in enzymes that synthesize sphingolipids, which are conserved lipids that regulate critical processes (e.g., actin cytoskeleton dynamics in yeast to inflammation in humans) and that are altered in diseases of aging (e.g., cancer, heart disease). Specifically, we observed age-dependent increases in the levels of Lcb1p (endoplasmic reticulum (ER) localized subunit of long-chain base (LCB) synthase) and Lcb4p (cortical ER LCB kinase). We also observed a striking, age-dependent aggregation of Lac1p (ER ceramide synthase). In addition, we observed reduced levels of Lag1p (cortical ER ceramide synthase). Interestingly, an age-associated decrease in the levels of LAG1 (Longevity Assurance Gene 1) mRNA and an extension of lifespan of lag1 cells were among the first changes reported in yeast aging[1]. Because Lac1p and Lag1p encode the same enzymatic activity, we asked whether lag1 affected the aggregation of Lac1p. We found that lag1 delayed the age of onset of Lac1p aggregation. Overall, we identified specific age dependent changes that occur in the distribution and levels of enzymes involved in sphingolipid synthesis, which may impact sphingolipids levels and signaling as well as lifespan. Studies in progress will be used to assess what underlies the changes identified above, which may provide insights into changes that occur in lipid metabolism with age and potentially how the aging process contributes to disease in humans. [1]Dmello NP, Childress AM, Franklin DS, Kale SP, Pinswasdi C, Jazwinski SM. 1994 J Biol Chem 269 (22): 15451-15459.

189C. Molecular switches of Golgi size control. ***Madhura D. Bhave***, *Prasanna Iyer, Bhawik Jain, Dibyendu Bhattacharyya*. Bhattacharyya lab, Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Sector 22, Navi Mumbai-410 210. MH INDIA.
How intracellular organelles can regulate their size is a very fundamental question and largly unknown. Previously from our lab we have shown that ARF1 controls the Golgi size by altering the maturation kinetics of Golgi cisternae in budding yeast. The rate of influx and efflux of membrane plays important role in regulation of size of Golgi apparatus. We have developed a mathematical model and simulation for understanding the dynamics of Golgi cisternae; taking into account how altering the rate of fusion of early Golgi cisternae and fission from late Golgi cisternae can affect the Golgi cisternae

size. Along with Arf1 & other GTPases, specially Rab GTPases have important regulatory roles in vesicle trafficking. We are studying knock out or knock down effect of wide range of GTPases on Golgi size by comparing the 4D microscopic data of cisternal dynamics. Systematic study of Rab and other Golgi associated GTPases will lead to detailed understanding of how altering cisternal maturation kinetics can affect Golgi cisternae size.

190A. The osmotolerant yeast *Zygosaccharomyces rouxii* possesses two differently regulated glycerol transporters. ***Michala Bubnová***[1], *Hana Sychrová*[1], *Candida Lucas*[2]. 1) Department of Membrane Transport, Institute of Physiology Academy of Sciences of the Czech Republic, v.v.i, Prague, Czech Republic; 2) Departement of Biology, University of Minho, Campus de Gualtar, Braga, Portugal.
Glycerol is the main osmoprotectant in most yeast species and is accumulated and produced at high quantities especially under hyperosmotic conditions. The osmotolerant yeast species (e. g. *Zygosaccharomyces rouxii*, *Debaryomyces hansenii*) are distinguished by a more efficient glycerol uptake system, which helps them to reach necessary intracellular concentration with relatively low glycerol production. *Saccharomyces cerevisiae* possesses two systems for glycerol transport. First of them is Fps1, a plasma-membrane channel that is required for a quick release of glycerol. Second transporter, Stl1, mediates active uptake of glycerol in symport with protons and is repressed and inactivated by glucose in *S. cerevisiae*. In the genome of *Z. rouxii*, we have found two putative orthologues of the *S. cerevisiae STL1* (*ZrSTL1* and *ZrSTL2*) and our results confirmed that both genes encode plasma-membrane glycerol transporters. Mutants lacking *ZrSTL1* and/or *ZrSTL2* are unable to grow under high osmotic pressure. Using the GFP-tagging, we localized *Zr*Stl1 and *Zr*Stl2 in the plasma membrane of *Z. rouxii*. The comparison of accumulation ratios of radiolabelled glycerol in *stl1/2* mutants and in the wild type shows that only double mutant does not accumulate any glycerol. Our RT-qPCR data reveals that fructose is the main regulator of glycerol uptake in *Z. rouxii* and that the expression of only *ZrSTL1* is strongly influenced by different carbon sources. *Zr*Stl2 plays a role of the housekeeping glycerol transporter. The H^+-symport mechanisms of both transporters is confirmed by the measurement of intracellular pH. Data acquired from the heterologous expression of *ZrSTL1/2* in *S. cerevisiae stl1fps1* suggest that only *ZrSTL1* is functional in *S. cerevisiae*. The estimated kinetic parameters (Km and Vmax) indicate that *Zr*Stl1 is a transporter with similar capacity as *Sc*Stl1.
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191B. A conserved role for GSK3 [illegible] lipin homeostasis through lipins.
Leslie J. Chan[1,5], *Jennifer McQueen*[1,3,5], *Timothy R. Peterson*[2,5], *Meredith Briggs*[1], *David M. Sabatini*[2,4], *Vivien Measday*[3], *Christopher J.R. Loewen*[1]. 1) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada; 2) Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA; 3) Wine Research Centre, Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada; 4) Howard Hughes Medical Institute; 5) These authors contributed equally to this work.
Despite the increasing prevalence of lipid-associated diseases such as obesity and diabetes, we lack a comprehensive understanding of the cellular regulators of lipid homeostasis. The lipid phosphatidic acid (PA) is a precursor in the synthesis of both phospholipids and neutral lipids, and is therefore positioned at a key intersection point between cell growth and fat storage. Lipins are conserved PA phosphatase enzymes that convert PA to diacylglycerol and exert significant influence on lipid homeostasis by mediating cellular neutral lipid levels, and through regulation of SREBP-dependent target gene expression. Phosphorylation has been found to be a major mode of lipin regulation, however, to date only the mTOR kinase has been found to directly regulate human Lipin-1. Here we report the results of an unbiased genome-wide synthetic dosage lethality screen (SDL) for kinase regulators of the yeast lipin, Pah1. Our screen identified 11 kinases that showed SDL interactions with Pah1. We further define a novel role for the yeast glycogen synthase kinase (GSK3) homologue Mck1 as a negative regulator of Pah1. Mck1 null yeast cells exhibited increased Pah1 activity and increased neutral lipid accumulation in lipid droplets. Furthermore, we provide both *in vivo* and *in vitro* evidence to support a direct role for Mck1 in Pah1 phosphorylation. We show that mammalian GSK3 is capable of phosphorylating Lipin-1, and inhibition of GSK3 decreases SREBP target gene expression, consistent with repression of Lipin-1 by GSK3. Our work emphasizes the power of yeast screening to identify novel regulators of lipid homeostasis in humans.

192C. Characterization of *Candida glabrata* growth and cell wall enzymes in during stress. *Yuanyuan Chew*, ***Cheen Fei Chin***, *Foong May Yeong.* Biochemistry, National University of Singapore, Singapore, Singapore.
Candida glabrata, previously known as *Torulopsis glabrata*, is a haploid yeast lacking mating activity that grows only as a yeast form. *C. glabrata* is generally accepted to be a commensal fungus that is part of the microbial flora of the mucosal surfaces of the human body. However, *C. glabrata* can also exist as an opportunistic pathogen, especially in immuno-compromised individuals. *C. glabrata* belongs to the Non-Candida albicans species of fungal pathogens that is gaining increasing clinical significance as it has been shown to be resistant to echinocandins, an important group of anti-fungal drugs known to inhibit synthesis of the major fungal cell wall polysaccharide (1,3)-glucan.
Based on previous studies, it is thought that the chitin levels in the *C. glabrata* cell wall remain unchanged when the cells were stressed due to exposure to echinocandins. In this study, we characterized cell viability and cell wall enzymes from

C. glabrata (CBS138, ATCC 2001) to further understand changes in survival and regulation of cell wall synthesis during treatment of cells with various stress factors. The various stress factors include nutrient starvation, oxidative stress and low pH that could be physiologically significant to the yeast cells during pathogenesis.
Data from our study provided insights into the growth of *C. glabrata* in different environmental conditions. We also report here our findings using Real-time PCR and Western blot analysis on the levels of cell wall transcripts and enzymes respectively. In addition, we present our initial observations on how stress factors can play a role in altering cell wall synthesis based on our study using cell wall stains detected via microscopic examination of cells. A deeper understanding of cell survival and cell wall regulation could contribute to the current knowledge of *C. glabrata* that is an emerging fungal pathogen.

193A. The JmjC Domain-containing Regulator Gis1 is Regulated by Levels of Heme and Oxygen Independently.
***Jonathan M Comer**, Ajit Shah, Sneha Lal, Thai Cao, Jagmohan Hooda, Li Zhang.* Molecular and Cell Biology, University of Texas at Dallas, Richardson, TX.
Heme plays key regulatory roles in the sensing and utilization of oxygen in fundamental molecular and cellular processes in all living organisms, ranging from bacteria to humans. The CP motif of some proteins binds heme reversibly, facilitating regulation of protein function. Gis1 is a unique transcriptional regulator belonging to the JHDM3/JMJD2 subfamily of demethylases containing CP motifs. The CP motifs of Gis1 reside in its JmjC domain and C2H2 type zinc finger; Gis1 also contains a JmjN domain, a coiled-coil domain, and two transcription activation domains. In S. cerevisiae Gis1 is responsible for the induction of genes after glucose depletion, through a PDS motif. Previously in our lab, fluorescent live cell imaging of Gis1 showed that it localizes to the nucleus in normoxic cells and to the cytosol in hypoxic cells. Analysis using the PDS-LEU2-lacZ reporter gene showed a significant increase in Gis1 transcriptional activity under high heme concentration. However, hypoxic conditions led to a relative decrease in Gis1 activity. These results indicate that Gis1 activity is regulated by both oxygen and heme, and that oxygen and heme work together to regulate Gis1 activity. Various domain deletions of Gis1 were studied in order to determine the roles these domains play in regulating Gis1 transcriptional activity. These experiments showed that Gis1 response to heme may involve more than one heme binding site. Purification of Gis1 followed by biochemical assays indicate that Gis1 binds heme directly, and preliminary studies of purified Gis1 domain deletions support the idea that there is more than one heme binding site. Further studies will determine the locations and biochemical nature of heme binding to Gis1.

194B. Characterization of the CIA complex in maintaining genome stability.
***Lilach Emuna**, Marina Volpe, Galit David Kadoch, Shay Ben A. roya.* Faculty of Life Science, Bar-Ilan University, Ramat-Gan, Israel.
Chromosome Instability (CIN) is a preliminary condition for the development of cancer. To expand the known spectrum of genes that play a role in maintenance of genome stability, the whole yeast genome was screened for a CIN phenotype. The screen results reveal proteins involved in the mitochondrial and cytosolic Fe-S cluster biogenesis. The functional distribution of the identified genes suggested several unexpected pathways such as iron-sulfur (Fe-S) biogenesis. This vital and evolutionary conserved pathway uses Fe-S clusters as a cofactor for multiple proteins involved in DNA replication and repair. The maturation of the cytosolic iron-sulfur proteins is carried out by the cytoplasmic iron-sulfur protein assembly (CIA) machinery. The aim of this project is to identify and characterize new iron-sulfur proteins which are required for maintaining genome stability. We show that the CIA complex co-localizes with components of the mitotic pathway such as tubulins at spindle pole body, and the midbody matrix surrounding the compacted midzone microtubules during cytokinesis. These results may suggest for the first time that mitotic proteins are a target of CIA machinery. We propose that Kif4A is one of the potential targets of the CIA complex. Kif4a is a chromokinesin that localizes to midzone and midbody during cytokinesis and suppresses microtubule dynamics. Our results indicate that Kif4A interacts with CIA complex members and its levels are reduced under CIA complex depletion. Moreover, KIF4A carries cystein rich domain which is responsible for its localization to the midbody and for the interaction with the CIA complex. Preliminary biochemical assays reveal 55Fe incorporation into the cystein rich domain of KIF4A. We believe that Fe-S modification on mitotic proteins plays a crucial role in regulating proper chromosome segregation.

195C. [PSI+], a prion-containing yeast cell, can switch into agar-invasive growth while [psi-] ,a non-prion strain cannot.
***Irene M. Evans**, Ying Peng Lee, Prashanti Patil, Haeja A. Kessler, Dylan S Weil, Brandy A Dennis.* Irene M. Evans, Rochester Inst Tech, Rochester, NY 14623.
Yeast prions are inheritable, infectious, self-propagating protein particles that may provide an epigenetic mechanism for promoting heritable diversity. Prions may promote survival in fluctuating stressful environments by allowing the yeast that harbor them to switch into pseudohyphal filamentous growth and migrate to find new food sources. Yeast cells enter different differentiation pathways according to nutrient availability and environmental conditions. Our hypothesis is that [PSI+], a prion-containing strain, can switch to filamentous pseudohyphal growth under stressful conditions while [psi-], a prion-negative strain, cannot. In order to check whether the strains are invasive/non-invasive under different stressful conditions, we tested the strains by pre-growing them in ornithine medium and then using three different invasion assays,

two of which are novel and developed in our laboratory. [PSI+] and [psi-] strains were tested for invasive growth using 1) an agar washing assay, 2) a test tube invasion assay, or 3) a confocal invasion assay. Upon prior exposure to nitrogen-limiting medium conditions, [PSI+], a Sup35 prion-containing cell, enters pseudohyphal growth and invades when plated on YPD agar. This result was observed in all three agar invasion assays. [psi-], which contains Sup35 in its native functional form, was not invasive in the confocal invasion assay. However, in recent experiments, when [psi-] is grown under the same nitrogen-limiting conditions and then plated on YPD agar, some invasion is observed in the test tube agar invasion assay. When invasion is observed using [psi-], the invading cells were white in color suggesting the [psi-] phenotype may have converted to [PSI+] and prion switching may have occurred. [PSI+] prion-containing strains are able to read through stop codons and this ability of [PSI+] may explain our results. If a stop codon has occurred in a gene required for pseudohyphal growth, then [PSI+] may read-through the stop codon and produce the protein and thus allow agar invasion.

196A. The Role of Phosphorylation in Ribosomal Protein L4. ***Jesse Michael Fox***, *Lasse Lindahl.* Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.
The Saccharomyces cerevisiae large ribosomal subunit consists of three ribosomal RNAs (5.8S, 25S, and 5S) and 54 ribosomal proteins, and the small subunit consists of one ribosomal RNA (18S) and 36 ribosomal proteins. These components temporally bind to one another to form the mature ribosome during ribosome biosynthesis. At the beginning of ribosome biosynthesis, the 18S, 5.8S and 25S ribosomal RNAs are transcribed as a single transcript. This transcript is then cleaved, modified, and transported from the nucleolus to the cytoplasm, a complicated process that requires endo and exo-nucleases and over 200 ribosome modification and assembly factors. The ribosomal proteins gradually bind to the maturing ribosomal RNAs throughout this process eventually forming the mature subunits in the cytoplasm. Given the abundance of phosphorylation in most cellular processes, it is not surprising to find over 300 phosphorylation sites in the ribosomal proteins (PhosphoPep and PhosphoGRID Databases). Ribosomal large subunit protein 4 (L4) is the most highly phosphorylated ribosomal protein with 15 highly confident phosphorylation sites. Four of these are conserved between yeast and mice. To date there have been very few studies about the function of phosphorylation on ribosomal proteins. The most studied phosphorylated ribosomal protein is the ribosomal small subunit protein 6 and it was determined that phosphorylation caused a higher translation rate. Here, we report mutations in all 15 phosphorylated serine and threonine sites in ribosomal protein L4, including one which all phosphorylation targets are mutated to alanine. We determine with the 15 alanine mutant, L4 phosphorylation is not necessary for viability, but it does cause a slight growth defect. We also identified that a phosphomimetic mutation at threonine 129 causes a defect in ribosome biogenesis. More specifically, it significantly slows the A3 to B1 cleavage forming the mature 5 end of the 5.8S ribosomal RNA. These results suggest, phosphorylation in the ribosomal protein L4 is not necessary, but could be a way to transiently stop or slow ribosome production during times of distress or to optimize ribosome biogenesis.

197B. Understanding the role of the translation factor eIF5A through genetic interaction network of different mutants. ***Fabio C. Galvao***[1*], *Sara Sharifpoor*[2], *Danuza Rossi*[1], *PPaulo E. G. Boldrin*[1], *Natalia M. Barboas*[1], *Brenda J. Andrews*[2], *Cleslei F. Zanelli*[1], *Sandro R. Valentini*[1]. 1) Biological Science, UNESP-Univ. Estadual Paulista, Araraquara, São Paulo, Brazil; 2) Department of Molecular Genetics, The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto.
The eukaryotic translation factor 5A (eIF5A) is highly conserved from Archaea to mammals and essential for cell viability. It is the only protein known to contain the unique and essential amino acid residue hypusine, generated through a posttranslational modification known as hypusination. Although eIF5A has been recently described as a translation elongation factor, its specific role in this process and the role played by its hypusine residue are still not known. To address these questions, we performed Synthetic Genetic Array (SGA) analyses using four temperature-sensitive eIF5A mutants (tif51A-1, tif51A-3, tif51AK56A and tif51AQ22H/L93F), which produce stable or unstable eIF5A proteins with different phenotype severity, and a yeast haploid deletion mutant collection, a temperature-sensitive mutant collection or a GST-fusion protein overexpression collection. The preliminary data analysis revealed interactions with genes that are already known to interact with the gene encoding eIF5A, validating the SGA analyses performed. Furthermore, Gene Ontology analysis revealed several interactions with genes encoding proteins involved in the secretory pathway and translation/protein synthesis, consistent with the involvement of eIF5A in both of these cellular processes. Interestingly, genes encoding proteins with polyproline tracks were also revealed in agreement with the recent hypothesis that eIF5A might be a factor responsible for the translation of polyproline track-containing proteins. The results obtained in the SGA analyses are been confirmed and further investigation will contribute to the understanding of the precise function of eIF5A. Supported by: FAPESP, CNPq and PADC.

198C. Allele-specific SGA screening to identify functions for ER polarization. ***Analise K. Hofmann***, *Andrew K.O. Wong, Christopher J.R. Loewen.* 2350 Health Sciences Mall University of British Columbia Vancouver, B. C. Canada V6T 1Z3.
Budding yeast is an excellent model for studying eukaryotic cell polarity because it grows vegetatively by asymmetric

budding, creating a polarized cellular domain that becomes a new daughter cell with each round of the cell cycle. We discovered that interaction of an ER protein Scs2 with a septin Shs1 at the bud neck creates a diffusion barrier on the ER membrane that polarizes the ER into separate bud and mother diffusion domains. To uncover physiological functions for ER polarization we performed SGA screens with point mutants of Scs2 that disrupted binding to Shs1 by transforming a SCS2 null query strain with individual plasmids containing each mutant. Using colony analysis software developed in our lab called Balony we identified genes that showed aggravating/negative genetic interactions with each Scs2 point mutant. Allele-specific SGA analysis identified genetic interactions corresponding to known functions for ER polarization in S phase spindle positioning as well as novel interactions.

199A. YlSnf1 Affects the Production of Omega-3 Fatty Acids from Yarrowia lipolytica. *J. Seip, R. Jackson, H. He, Q. Zhu,* ***S-P. Hong***. Biotechnology, DuPont Central R&D, Wilmington, DE.
EPA and DHA omega-3 fatty acids are essential nutrients in the diets of humans and animals. The current major source of EPA and DHA is fish oil. However, concerns over the quality and sustainability of the fish oil supply have generated interest in supplying omega-3 fatty acids from alternative sources. We developed a metabolically engineered strain of the oleaginous yeast Yarrowia lipolytica. In Y. lipolytica, de novo lipid synthesis and accumulation are induced under conditions of nitrogen limitation (or a high carbon-to-nitrogen ratio). The regulatory pathway responsible for this induction has not been identified. Here we report that the SNF1 pathway plays a key role in the transition from the growth phase to the oleaginous phase in Y. lipolytica. Strains with a Y. lipolytica snf1 (Ylsnf1) deletion accumulated fatty acids constitutively at levels up to 2.6-fold higher than those of the wild type. In a EPA production strain, Ylsnf1 deletion led to a 52% increase in EPA titers (7.6% of dry cell weight) over the control. Other components of the Y. lipolytica SNF1 pathway were also identified, and their function in limiting fatty acid accumulation is suggested by gene deletion analyses. Deletion of the gene encoding YlSnf4, YlGal83, or YlSak1 significantly increased lipid accumulation in both growth and oleaginous phases compared to the wild type. Furthermore, microarray and quantitative reverse transcription-PCR (qRT-PCR) analyses of the Ylsnf1 mutant identified significantly differentially expressed genes during de novo lipid synthesis and accumulation in Y. lipolytica. Gene ontology analysis found that these genes were highly enriched with genes involved in lipid metabolism. These studies show new roles for Snf1 in lipid accumulation in this oleaginous yeast.

200B. PM - ER Membrane Tethering Complexes and Non-vesicular Sterol Transport. ***Jesper Johansen***[1]*, Evan Quon*[1]*, Yves Sere*[2]*, Anant K Menon*[2]*, Christopher T Beh*[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Colombia, Canada; 2) Department of Biochemistry, Weill Cornell Medical College, New York, NY.
Intracellular trafficking of sterols occurs largely by a poorly understood non-vesicular transport process, which operates independently of vesicular pathways that transport secretory proteins. Two models have been proposed for how sterols, like cholesterol and yeast ergosterol, move from their site of synthesis in the endoplasmic reticulum (ER) to where they reside in the plasma membrane (PM). One model proposes that cytoplasmic sterol transport proteins (STPs) ferry sterols between membranes. Another possibility is that sterol transfer occurs directly between the PM and ER at sites where the membranes are closely associated (membrane contact sites or MCSs). Although yeast STPs have not been identified, based on in vitro lipid binding and transfer assays the cytoplasmic protein Osh4/Kes1p has been implicated in the phosphoinositide-driven cycling of sterols between ER and Golgi membranes. In addition, other members of the Osh protein family have functions at PM-ER MCSs involving phosphoinositide metabolism, prompting the hypothesis that the Osh protein family members function as STPs. Using a combination of genetic, microscopic and sterol transfer assays we tested whether Osh proteins are required in vivo for non-vesicular sterol transfer to the PM, and if PM-ER MCSs are required for sterol transport between the two membranes. We find that reductions in sterol levels increase PM-ER membrane association by increasing the density of MCS tethering complexes. However, despite the sterol-dependent control of MCSs, the elimination of PM-ER MCS tethering complexes had no impact on sterol traffic either to or from the PM. These findings are contrary to the presumed role of Osh proteins and PM-ER MCSs in non-vesicular sterol transport, which is not supported by experimental data when tested in vivo.

201C. Hsp104 as stress indicator in hybrids of the Saccharomyces sensu stricto complex. ***Claudia Kempf****, Jürgen Wendland.* Carlsberg Laboratory, Copenhagen, Denmark.
The Saccharomyces sensu stricto complex is a group of different yeast species, which are used in fermentation processes. This group includes S. cerevisiae, S. bayanus, S. paradoxus, S. mikatae, S. kudriavzevii and S. cariocanus. The members of this group are closely related and are able to mate and produce viable hybrids. The hybrids propagate by mitotic divisions, but produce only few viable spores. Hybridization between two different species forms a hybrid harboring two genomes, which expresses both proteomes and thus harbor characteristics of both parental strains. The expression of both proteomes will lead to the formation of hybrid protein complexes which could affect the function of these complexes and thus generate hybrid specific responses to environmental changes. We generated diploid hybrids of S. cerevisiae and other members of the Saccharomyces sensu stricto group and analyzed their stress tolerance. For this we employed a chromosomally integrated ScHSP104-GFP as an in vivo stress indicator. Hsp104 is a heat shock protein, which localizes

to the cytoplasm and the nucleus in unstressed cells. Under different stress conditions Hsp104 binds to denatured and aggregated proteins to promote their refolding. These aggregates are visible as HSP104-GFP foci in the cell and the number of formed foci can be used as an indicator of the stress level in the different hybrids. We present results of heat-stressed and ethanol stressed cells that show differential responses of these hybrids to environmental stresses.

202A. Roles of the Yap1 Transcription Factor and Antioxidants in *Saccharomyces cerevisiae*'s Tolerance to Furfural and 5-Hydroxymethylfurfural, which Function as Thiol-Reactive Electrophiles Generating Oxidative Stress. ***Daehee Kim***, *Ji-Sook Hahn.* Chemical and Biological engineering, Seoul National University, Seoul, South Korea.
Development of the tolerance of *Saccharomyces cerevisiae* strains to furfural and 5-hydroxymethylfurfural (HMF) is an important issue for cellulosic ethanol production. Although furfural and HMF are known to induce oxidative stress, the underlying mechanisms are largely unknown. In this study, we show that both furfural and HMF act as thiol-reactive electrophiles, thus directly activating the Yap1 transcription factor via the H_2O_2-independent pathway, depleting cellular glutathione (GSH) levels, and accumulating reactive oxygen species in Saccharomyces cerevisiae. However, furfural showed higher reactivity than did HMF toward GSH in vitro and in vivo. In line with such toxic mechanisms, overexpression of $YAP1^{C620F}$, a constitutively active mutant of *YAP1*, and Yap1 target genes encoding catalases (*CTA1* and *CTT1*) increased tolerance to furfural and HMF. However, increasing GSH levels by overexpression of genes for GSH biosynthesis (*GSH1* and *GLR1*) or by the exogenous addition of GSH to the culture medium enhanced tolerance to furfural but not to HMF.

203B. A metabolic strategy to enhance long-term survival by Phx1 through stationary phase-specific pyruvate decarboxylases in fission yeast. ***Eun Jung Kim***, *Ji Yoon Kim, Jung Hye Roe.* School of Biological Sciences, Seoul National University, Seoul, Korea.
In Schizosacchromyces pombe, Phx1 (pombe homeobox) transcription factor is expressed at stationary phase, regulating the long-term survival, stress resistance, and meiosis. We identified Phx1-dependent genes through transcriptome analysis, and further analyzed those related with carbohydrate and thiamine metabolism, and whose expression decreased in phx1. The level of thiamine pyrophosphate (TPP) and TPP-utilizing pyruvate decarboxylase activity that converts pyruvate to acetaldehyde were reduced in the phx1 mutant. Therefore, Phx1 is thought to be involved in shifting metabolic flux by diverting pyruvate from TCA cycle and respiration to ethanol fermentation. Among the four putative genes for pyruvate decarboxylase, pdc201+ and pdc202+ were Phx1-dependent, contributing to long-term survival. We therefore propose that Phx1-dependent lifespan extension is achieved through increasing the synthesis and activity of pyruvate decarboxylase. Consistent with this hypothesis, we observed that Phx1 curtailed respiration when cells entered stationary phase. Pka1 and Sck2 are known to regulate the lifespan as the pro-aging kinases of nutrient signaling pathway. Introduction of phx1 mutation compromised the long-lived phenotype of pka1 and sck2 mutants that are devoid of pro-aging kinases of nutrient signaling pathway. It also diminished the lifespan of pyp1 mutant which constitutively activates stress-responsive kinase Sty1. Therefore, achievement of long-term viability through both nutrient limitation and anti-stress response appears to be dependent on Phx1.

204C. Evaluation of cytotoxicity caused by the strong expression of GFPs with various localization signals. ***R. Kintaka***, *K. Makanae, H. Moriya.* RCIS, Okayama University, City of Okayama, Japan.
Overexpression of more than 20% of proteins causes growth defect in S. cerevisiae (Sopko et al. 2006, Makanae et al. 2013). Although some molecular mechanisms causing the growth defect upon protein overexpression are proposed (Tong and Amon 2013), the reason why these proteins cause growth defect are largely unknown. We previously reported a method designated genetic tug-of-war (gTOW), by which we can evaluate the limit of protein expression to halt cellular growth (Moriya et al, 2006). In gTOW we use the limit copy number of the gene on a 2-based multicopy plasmid to estimate the limit of protein limit. Recently, we measured the expression limit of green fluorescent protein (GFP) as a model protein that is gratuitous in S. cerevisiae to measure the burden for protein turnover (Makanae et al. 2013). In this study, by expanding above experiment, we tried to estimate the influences of protein localization and protein aggregations using modified GFPs attached with carious localization signals, misfolding mutations, and poly-glutamate stretch. We attached; secretory signal sequence (SS), mitochondrial targeting sequence (MTS), nuclear export signal (NES), nuclear localization signal (NLS), cytoplasmic membrane anchoring signal (CC), degradation signal (DEG), misfolding mutation (m3), and 96 poly-glutamate stretch (Q96). These modified GFPs were expressed from PYK1 promoter. GFP with SS, MTS, NES, and m3 showed much lower expression limits than that of GFP alone. Although Q96 are reported to be toxic in S. cerevisiae (Park et al. 2013), the limits were higher than them. We observed aberrant morphologies of the cells upon overexpression of the modified GFPs. Cells expressing some of modified GFPs showed different cell cycle defects, such as prolonged G1 phase and nuclear division defect. To further characterize the physiologies upon overexpression of modified GFPs, we are currently performing transcriptome analysis, suppressor screening, and the same analyses using other proteins. In conclusion, the strong expression of proteins with localizations signals are cytotoxic as the same level of

misfolding protein, and caused various cellular dysfunctions such as cell cycle defects. Similar cytotoxicity might also cause the growth defect when native proteins were overexpressed.

205A. Swi3, a novel regulator of aerobic respiration genes and oxygen metabolism in *Saccharomyces cerevisiae*. ***Sneha Lal****, Jagmohan Hooda, Md Maksudal Alam, Ajit Shah, Thai Cao, Li Zhang.* Molecular and Cell Biology, University of Texas at Dallas, Richardson, TX.
Aerobic cellular respiration is vital for energy generation in eukaryotes ranging from yeast to humans. Previous studies in our lab with *Saccharomyces cerevisiae* have shown that many target genes of SWI/SNF components are oxygen-regulated, and hence may play an important role in the regulation of aerobic respiration and cellular energy production. Preliminary work in our lab indicates that Swi3 is a novel regulator that modulates aerobic respiration and oxygen metabolism. Fluorescent live cell imaging showed that oxygen is required for the nuclear localization of Swi3, but not for Swi2. We see elevated levels of oxygen consumption in *swi3* cells, whereas *swi2* cells show a similar rate of oxygen consumption as the wild type cells. Moreover, the promoter activities of aerobic respiration genes, CYC1 and CYC7, were dramatically increased by *swi3*. Analysis of the mitochondrial respiratory chain complex proteins by Blue Native (BN) polyacrylamide gel electrophoresis shows higher levels of expression in *swi3* as compared to *swi2* and the wild type cells. The human homologues of Swi3, namely, BAF155 and BAF170, which have been identified as tumor suppressors are also shown to regulate cellular respiration in various types of cancer cells. Genome-wide ChIP-Seq data in HeLa cells shows that BAF155 and BAF170, but not Brg1 (Swi2 homolog), are associated with many genes encoding functions required for oxidative phosphorylation. Further, we show that the rate of oxygen consumption in HeLa cells increases when BAF155 or BAF170 is knocked down. Collectively, these results support the idea that Swi3 and its human homologues play an important role in moderating aerobic respiration gene expression and oxygen metabolism. Further, we are investigating the mechanism of oxygen sensing by Swi3 and its human homologues.

206B. Mechanism of Non-Genetic Heterogeneity in Growth Rate of *Saccharomyces cerevisiae*. ***Shuang Li****, Mark Siegal.* Department of Biology, New York University, New York, NY.
Despite its genetic homogeneity, a clonal population can display marked heterogeneity in various aspects. This non-genetic heterogeneity is not only theoretically interesting, but also clinically important, because of its relevance to cancer and microbial antibiotic resistance. A clonal yeast population growing in benign conditions contains cells growing at different rates. The slow-growing ones, which show increased expression of TSL1, a subunit of the trehalose synthase complex, disproportionately survive acute heat shock (Levy, S.F. et al. 2012, PLoS Biology). Growth-rate heterogeneity can be viewed as a bet-hedging strategy, whereby a clonal population maximizes its long-term success by diversifying its phenotypes. However, the mechanisms generating this heterogeneity are poorly understood. With a novel high-throughput microcolony imaging system and genetic analysis, we have investigated the involvement of the MSN2/4 pathway in regulating growth-rate heterogeneity. MSN2 and MSN4 are transcription factors that regulate the general stress response. The phosphorylation status of MSN2 regulates its nuclear import and export rates, which in turn determine its pattern of subcellular localization (Hao, N. et al. 2013, Science). Although there have been extensive studies on the function and localization dynamics of MSN2 under stress, the significance of MSN2 under benign conditions has rarely been explored. Analysis of individual microcolony development shows that knocking out either MSN2 or MSN4 leads to significant reduction of slow-growing individuals. Restricting nuclear import of MSN2 and MSN4 by elevating Protein Kinase A activity recapitulates this population growth-rate distribution change. Heterogeneous TSL1 expression, and hence stress resistance, is also dependent on MSN2, but not on MSN4: knocking out MSN2 abolishes TSL1 heterogeneity by completely suppressing its expression, whereas knocking out MSN4 has only minor effects on TSL1 expression. Dynamics of MSN2 localization also relate to growth rate, in that slow-growing cells have significantly longer dwell times of MSN2 in the nucleus. These results suggest that variable activity of the MSN2/4 pathway under benign conditions causes heterogeneity of growth and stress resistance that may benefit clonal population survival.

207C. Heavy Water Promotes Longevity in Yeast. ***Xiyan Li****, Michael Snyder.* Genetics, Stanford University, Stanford, CA.
Normal aging is accompanied with chronic accumulation of biochemical anomalies. Since biochemical reactions can be greatly affected by heavy isotopes due to kinetic isotopic effect (KIE), one would expect that stable isotopes may influence aging at the biochemical level. Kinetic isotopic effect has been proposed, but never demonstrated to extend lifespan in any organism. In this study, we showed that deuterium, in the form of heavy water (D_2O), extended the lifespan of budding yeast *S. cerevisiae* in both chronological aging (by up to 85%) and replicative aging (by up to 28%). Heavy water extended the CLS independent of several known yeast lifespan regulator genes, including *SIR2* and *TOR1*. However, heavy water was unable to further extend the CLS in yeast under mild calorie restriction (0.5% glucose). Furthermore, CLS extension by heavy water was substantially attenuated in yeast lacking mitochondria, suggesting that deuterium exerts its effects mainly through mitochondrial metabolism. As organismal aging is closely associated with metabolic changes, our findings may lead to a novel strategy to promote longevity and expand current understanding of the chemical basis for aging.

208A. Characterizing SUMO Function in *Saccharomyces cerevisiae* Using a Versatile Library of Synthetic *SMT3* Mutants. ***P. B. Meluh***[1], *H. A. Newman*[2], *J. Lu*[2], *J. D. Boeke*[3], *M. J. Matunis*[2]. 1) Dept Mol Biol & Gen, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Dept. Biochem & Mol Biol, Johns Hopkins Bloomberg Sch Public Health, Baltimore, MD; 3) Dept Biochem & Mol Pharm, NYU Langone Univ Sch of Med, New York, NY.
Small ubiquitin-like modifier proteins (SUMOs) serve as reversible post-translational modifications that can affect protein localization, protein stability and protein-protein interactions. In many systems, including budding yeast, dynamic SUMOylation is essential for cell viability and implicated in many processes including DNA replication and repair, transcription, pre-mRNA processing, chromosome segregation and mitochondrial fission. To gain insight into SUMOs multi-functionality, we generated a systematic collection of 250 molecularly bar-coded yeast mutants, each containing a different missense or deletion mutation in *SMT3*, the sole gene encoding yeast SUMO. This mutant collection, either individually or as a pool, is currently being characterized phenotypically and biochemically. To date, we have identified amino acid residues essential for cell viability, as well as ones critical for survival under various stress conditions including temperature extremes, DNA damage, protein misfolding, oxidative and heavy metal stress. Not surprisingly, many residues thus identified localize to two regions on SUMO known to be important for interacting with either the conjugation and deconjugation machineries and/or proteins that contain a SUMO interacting motif (SIM). The N- and C-terminal extensions on SUMO are dispensable for viability, as well as for the response to stress. SUMO chain formation appears critical for the response to stress; however, linkage-specific chains with distinct functions like those seen with ubiquitin have not yet identified. This collection represents a rich resource for the community and should provide many starting points for biochemical and genetic screens. For example, several lethal alleles apparently defective for deconjugation might serve as useful *in vivo* "traps" for better defining the SUMO-modified proteome and genetic suppressors of these alleles might define additional pathway components and/or key SUMO substrates.

209B. Changes in transcription and metabolism during the early stage of replicative cellular senescence in budding yeast. ***Yukio Mukai***[1], *Yuka Kamei*[1], *Yoshihiro Tamada*[2], *Yasumune Nakayama*[2], *Eiichiro Fukusaki*[2]. 1) Department of Bioscience, Nagahama Institute of Bio-Science and Technology, 1266 Tamura-cho, Nagahama, Shiga 526-0829, Japan; 2) Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.
In senescent cells, age-related damage accumulates and a variety of biological activities and functions deteriorate. However, what cellular aging processes change, and when cellular aging behaviors begin are little known. While past research has demonstrated age-related mRNA changes in budding yeast by the 18^{th}-20^{th} generation, which is an average of replicative lifespan, about half of the population is dead by this time point. Here, we performed transcriptional and metabolic profiling for yeast at the early stages of senescence (4^{th}, 7^{th} and 11^{th} generation), that is, for populations in which almost all cells still were alive. Microarray expression analysis showed up- and down-regulation for about 20% genes after 4 generations, little further change by the 7^{th} generation, and additional 12% genes were up- and down-regulated after 11 generations. Pathway analysis revealed that these 11^{th} generation cells had accumulated transcripts coding for components of the sugar metabolism, tricarboxylic acid (TCA) cycle and amino acids degradation, and were depleted for mRNAs coding for components of amino acid biosynthetic pathways. These observations were consistent with the metabolomic profiles of 37 compounds of aging cells by gas chromatography-mass spectrometry. Aging cells exhibited accumulation of pyruvic acid and TCA cycle intermediates, and depletion of most amino acids, especially branched chain amino acids. High expression of stationary phase-induced genes (*SNO1*, *SNZ1* and *SPG4*) was observed after 11 generations. These genes also are induced by nutrient deprivation; however, we confirmed the growth medium used here was still not depleted for nutrients even after 11 generations of growth. Additionally, 15 members of the 24-gene *PAU* (seripauperin) family, which are known to be induced by anaerobiosis, were highly transcribed after 11 generations in spite of culturing yeast cells under aerobic condition. Thus, yeast cells exhibit deterioration of sensing and/or signaling of nutrient and oxygen by the 11^{th} generation. These changes are presumably early indications of replicative senescence.

210C. Changes in intracellular abundance and localization of *Saccharomyces cerevisiae* Hsp31p under various environmental stresses suggest its role in neutralizing the effects of oxidative stress insult. ***Urszula Natkanska***[1], *Adrianna Skoneczna*[2], *Marek Skoneczny*[1]. 1) Department of Genetics, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warszawa, Poland; 2) Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warszawa, Poland.
Hsp31p is a stress response protein belonging to ThiJ/PfpI family whose cellular function remains unknown. On the basis of numerous large scale analyses Hsp31p is characterized as low abundance cytosolic protein. We demonstrated previously that its abundance increases in response to oxidative stress and in the postdiauxic phase of growth. Continuing our functional analysis endeavor we investigated the response of this protein to various environmental stresses in terms of coding gene promoter activity, protein abundance and intracellular localization. Here we show that *HSP31* promoter activity strongly increases under various environmental stresses, including osmotic and metabolic stress. In most cases this agrees well with the increase of Hsp31p protein level. However under oxidative stress conditions there is considerably less

of Hsp31p in the cell than one could expect on the basis of *HSP31* promoter activity, which implies the accelerated turnover of this protein under oxidative stress. Interestingly, our test of Hsp31p localization with the help of Hsp31p-GFP fusion expressed from native genomic locus revealed that while in the cells growing exponentially in rich medium the protein is localized in the cytosol, in the same cells exposed to oxidative stress a part of the protein is found also in the cytoplasmic foci, similarly to several other stress response proteins including chaperones and peroxiredoxin Tsa1p. Taken together it is possible that Hsp31p participates in neutralizing the consequences of oxidative damage in the cell. Differential centrifugation combined with Western blotting with anti-Hsp31p antibodies confirmed cytosolic localization of native Hsp31p in exponentially growing cells, but failed to confirm the presence of particulate fraction of Hsp31p under oxidative stress conditions, probably due to the transient nature of Hsp31p-containing foci. Funding: Polish National Science Center grant no.: 2011/01/B/ NZ3/02904.

211A. Cell wall architecture in wine yeast: Compositional and physiological analysis of yeast cell walls having varying impact on wine protein stability. ***Thulile Ndlovu***, *Florian F Bauer*. Institute for Wine Biotechnology, Faculty of AgriSciences, South Africa, Western Cape, South Africa.
The removal of heat unstable grape proteins during white winemaking is a crucial step as cloudiness due to protein instability is unwanted in wine. Bentonite and other clarifying agents are used in industry to protect wine from protein haze, however these agents are costly and have undesirable impact on wine quality. Yeast mannoproteins released from the cell wall have been shown to play a crucial role in several technological, oenological and allergological processes due to their physicochemical properties. But so far none of the most commonly used wine yeast strains, all of the species Saccharomyces cerevisiae, has shown satisfactory activity under winemaking conditions with regards to wine clarification. Obtaining yeast strains that would naturally protect wine from haze formation would be highly desirable. In this study, following a screen of several yeast strains for their ability to reduce wine haze formation in Chardonnay and Sauvignon blanc must, an investigation of the yeast cell wall properties of haze protecting and non-haze protecting strains were carried out. Analytical and visual tools including Gas Chromatography-Mass Spectroscopy (GC MS), flow cytometry, confocal fluorescence microscopy and scanning electron microscopy were employed to study the physiological and chemical composition of the yeast cell wall. Yeast cell wall-protein binding assays were also carried out in order to elucidate the possible mechanism employed by the haze-protecting strains. Significant differences were observed in the yeast cell wall composition and properties. Moreover haze protecting strains bound more grape chitinase as well as commercial chitinases, enzymes that are thought to be primarily responsible for wine haze formation. This study clearly shows that cell wall properties of yeast can have a major impact on wine protein stabilization.

212B. Elucidating the Effects of Human Genetic Variation On Vitamin D Signaling. ***Lauren Richardson***, *Jasper Rine.* QB3, University of California, Berkeley, Berkeley, CA.
With the number and quality of human genome sequences increasing rapidly, connecting the variation between individuals to particular functional changes and phenotypes is the next major challenge. The goal of my research is to bridge the gap between the sequencing data and functional understanding, focusing on the vitamin D signaling cascade. Vitamin D signaling is critical to human health, and altered signaling has been implicated in numerous disease states, including cancer, autoimmune dysfunction, rickets, and several infectious diseases. Human genome sequencing projects, like the 1000 Genomes Project, have identified signal nucleotide polymorphisms (SNPs) within the vitamin D receptor (VDR) gene, yet whether and how these SNPs affect the function of VDR is relatively unknown. To tackle this problem, we will create a vitamin D signaling assay in yeast to determine if variants of VDR that exist in the population affect its ability to act as a transcription factor. This assay will allow us to screen variants in a high-throughput manner, while avoiding the endogenous negative feedback regulation of vitamin D. We will also determine if variants that abrogate vitamin D signaling are vitamin remedial by higher concentrations of vitamin D. After we have identified variants that impact VDR function, we will determine if they are able to alter a disease phenotype. Macrophages utilize vitamin D signaling to induce autophagy when they sense an infection, such as by Mycobacterium tuberculosis. I will create macrophage cell lines that exclusively express variant VDRs, and assess if these macrophages are capable of mounting autophagic responses to a simulated M. tuberculosis infection. By identifying VDR variants that abrogate the macrophage response to infection, we could identify individuals that have a genetically determined higher requirement for vitamin D.

213C. The stress response pathway is activated in *siw14* mutants. *Elizabeth Steidle, Daisy Walker*, ***Ronda J Rolfes***. Biology, Georgetown University, Washington , DC.
Yeast strains with mutations in *siw14* are defective in endocytosis, protein trafficking, stress responses, and linking nutrient status to cell cycle progression. Siw14 is a member of a family of dual-specificity phosphatases found in fungi, protists, and plants that is active with various phosphoinositides as substrates. We have begun to investigate the biochemistry of this enzyme as well as its effects on the cellular stress response. We have found up-regulation of the oxidative stress response as measured by resistance to exogenous hydrogen peroxide treatment. Furthermore, we observed a decrease in endogenous hydrogen peroxide but not superoxide levels. Mitochondrial function appears to be normal based

on growth on non-fermentable carbon sources and oxygen uptake. Strains also exhibit an increase in survival during stationary phase as measured by chronological aging assays. These experiments are consistent with the upregulation of the general cellular stress response as mediated by Msn2/Msn4.

214A. Inducible and rapid depletion of proteins in S. cerevisiae. ***Fabian Rudolf***, *Gintautas Vainorius, Moritz Lang, Joerg Stelling*. D-BSSE, ETH Zurich, 4058 Basel, Switzerland.
Purpose: To study the function of proteins selective gene inactivation and subsequent analysis of the phenotype is of great help. Classically, this was achieved by temperature sensitive alleles or the temperature dependent exposure of a degradation tag. Newer strategies include: site-specific mutations to accommodate a chemically modified ATP, anchor and away technique to omit the protein from functioning at specific locations, chemically induced recruitment to the proteasome or an E3-ligase or light induced display of a degron. To complement these tools and extend the possibilities, we constructed a two-component system for inducible and fast degradation of proteins able to be activated using different inducers. Methods: We used mathematical modeling, classical molecular biology techniques and protein engineering to construct the system. We monitored its performance using a combination of western blotting, flow cytometry, microscopy and plate and liquid based growth assays. We cloned the system in a vector to allow simple construction of N-terminal tagged genes driven by their own promoters. Results: Our system is based on an inducible split ubiquitin complementation as a mean to liberate a N-terminal degron and target the protein for destruction by the N-end rule pathway. We overcome the fast association rate of the two Ubiquitin halfs by destabilizing one thereby kinetically insulating the Ubiquitin complementation and minimizing the amount of uninduced cleavage of the target protein. Depletion of the protein of interest is assured by using either chemically or light inducible heterodimerization domains or in the simplest case by transcriptional induction of a constitutive heterodimerization domain. Using the rapamycin inducible FRB-FKBP domain dimerization, we optimized the system such that its performance is comparable to the chemical inhibition of the analogue sensitive Cdc28 allele. Further, we show, that we can inhibit Cdc28 function at the plasma membrane by selectively target one of the component there. Moreover, we tested the useability of our system by depleting a series of proteins localized in different compartments of the cell. Among those proteins are GTPases, kinases, pumps and transcription factors. Conclusion: Taken together, our split ubiquitin based protein degradation system provides a useful extension to the available gene inactivation technologies. It allows for rapid inactivation of protein function similar to the speed of chemical inhibiton, selective targeting of differently localized complexes and is controllable either by inducible promoters, chemicals or light.

215B. Changes in the sterol composition affect plasma-membrane potential, intracellular pH and the activity of MDR pumps in *Saccharomyces cerevisiae* cells. *Marie Kodedova*, ***Hana Sychrova***. Dept Membrane Transport, Inst Physiology AS CR, Prague 4, Czech Republic.
Proper plasma membrane composition is essential for yeast life. Especially sterol content is crucial for regulation of membrane permeability and fluidity, and for the regulation of activity of membrane transporters. We studied the impact of deletions of genes from the last steps in biosynthesis of ergosterol (*erg24, erg6, erg2, erg3, erg5, erg4*) on the physiological function of *S. cerevisiae* plasma membrane by a combination of the diS-C_3(3) fluorescence assay for the estimation of relative plasma-membrane potential, measurement of intracellular pH with the use of pHluorin, and biological tests. In comparison to the parental BY4741 strain, most of the *erg* mutants were more sensitive to a salt stress or cationic drugs, and the level of their sensitivity was connected to a relative hyperpolarization of their plasma membrane. The *erg* mutants (with the exception of *erg24*) had a lower intracellular pH than the parental strain and had problems to maintain the pH homeostasis upon NaCl stress. The observed higher sensitivity of *erg* mutants to cationic drugs resulted not only from the changed permeability of the plasma membrane and its relative hyperpolarization, but it was also due to a decreased activity of plasma-membrane MDR pumps. The strongest effect on MDR pumps activity was observed upon deletion of *ERG4* and *ERG6* genes. Altogether, our results suggested that the inhibition of activity of the Erg6 protein may become useful in the development of new antifungal drugs, as this C-24 methyltransferase is not involved in the cholesterol biosynthetic pathway. The work was supported within the project GA CR P503/10/0307 and the project The Center of Biomedical Research (CZ.1.07/2.3.00/30.0025) co-funded by the European Social Fund and the state budget of the Czech Republic.

216C. Selection for strains of Saccharomyces cerevisiae with enhanced Ochratoxin-A detoxification capabilities. ***Aaron Welch***. Chaplin School of Hospitality and Tourism, Florida International University, North Miami, FL.
The long-term goal of the proposed project is to study the mechanism of Ochratoxin-A toxicity, and create an inexpensive, simple, and powerful method of detoxifying Ochratoxin-A in wine-musts. Ochratoxin-A is one of the most common mycotoxins present in foods and beverages and is a threat to human health. It has been found in human plasma and milk and is classified as a possible carcinogen. It is produced by fungi such as Aspergillus carbonarius that grow on fruits and grains in warm environments throughout the world. With projected increases in global temperature, more winemaking regions are predicted to become hospitable environments to these Ochratoxin-A-producing fungi, which would cause an

increase in the frequency and intensity of Ochratoxin-A contamination in wines. The current methods used for removal of Ochratoxin-A from wines are inefficient, or unusable in a commercial winery. We propose a novel method to detoxify Ochratoxin-A-contaminated wines through directed-evolution of a strain of the wine-yeast Saccharomyces cerevisiae to select for robust OTA-detoxification activity. We believe that this is an ideal solution to an important human health problem. In the course of developing this strain of yeast, we will also gain insight into the mechanism of Ochratoxin-A induced toxicity, resistance, and detoxification.

217A. Identifying biomarkers of extended chronological lifespan through comparative gene expression profiling.
Margaret B Wierman, *Mirela Matecic, Veena Valsakumar, Daniel L. Smith, Stefan Bekiranov, Jeffrey S. Smith.* Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA.
The chronological lifespan (CLS) of Saccharomyces cerevisiae is defined as the number days that non-dividing cells remain viable, typically in stationary phase cultures or in water. CLS is extended by restricting glucose in the starting cultures, and is considered a form of caloric restriction (CR). Through a previous genetic screen our lab determined that deleting components of the de novo purine biosynthesis pathway also significantly increased CLS. Significant similarities in gene expression profiles between calorie restricted WT cells and a non-restricted ade4 mutant suggested the possibility of common gene expression biomarkers of all chronologically long lived cells that could also provide insights into general mechanisms of lifespan extension. We have identified additional growth conditions that extend CLS of WT cells, including supplementation of the media with isonicotinamide (INAM), a known sirtuin activator, or by supplementation with a concentrate collected from the expired media of a calorie restricted yeast culture, presumably due to an as yet unidentified longevity factor. Using these varied methods to extend CLS, we compared gene expression profiles in the aging cells (at day 8) to identify functionally relevant biomarkers of longevity. Nineteen genes were differentially regulated in all 4 of the long-lived populations relative to wild type. Of these 19 genes, viable haploid deletion mutants were available for 16 of them, and 12 were found to have a significant impact on CLS. Of particular interest was ADO1, which encodes adenosine kinase, and similarly to the de novo purine pathway, also produces AMP, suggesting a critical role for AMP/ADP levels in CLS regulation. Expression levels of the 19 common overlapping genes were also assessed in several unrelated long-lived deletion mutants to test as longevity biomarkers. Three genes (ADD66, FYV6, and LEU3) were found to be downregulated in the additional test strains, implying they can be used as future longevity biomarkers. Numerous gene expression changes were not shared by all four conditions, but instead clustered either between CR and ade4 or INAM and the CR concentrate, implying at least two distinct mechanisms behind the four respective CLS extensions, which are currently under investigation.

218B. An ER-Septin Diffusion Barrier Polarizes the Endoplasmic Reticulum. ***Andrew K.O. Wong***[1], *Jesse T. Chao*[1], *Shabnam Tavassoli*[1], *Barry P. Young*[1], *Adam Chruscicki*[2], *Nancy N. Fang*[2,3], *LeAnn J. Howe*[2], *Thibault Mayor*[2,3], *Leonard J. Foster*[2,3], *Christopher J.R. Loewen*[1]. 1) Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; 3) Centre for High-Throughput Biology, University of British Columbia, Vancouver, British Columbia, Canada.
Polarization of lipid membranes into domains is an important mechanism to compartmentalize cellular activities and to establish cell polarity. Polarization often requires formation of molecular diffusion barriers that prevent the lateral diffusion and mixing of proteins between such domains. The yeast bud neck, site of an hour-glass shaped septin collar, contains a diffusion barrier for both the plasma membrane and the endoplasmic reticulum (ER) membrane. This septin-dependent barrier compartmentalizes the yeast ER membrane into a bud and a mother domain, preventing ER membrane proteins from passively diffusing from the bud ER into the mother ER, and vice versa. We have recently shown that the molecular components of this diffusion barrier consists of the tail-anchored ER protein Scs2, a soluble subunit of the polarisome Epo1, and the septin Shs1. Physical interaction of these three components of the barrier is necessary to maintain compartmentalization of fluorescently-tagged ER membrane reporters to bud and mother compartments. Specific mutants that disrupt binding between barrier components results in a loss of compartmentalization of ER membrane proteins. Intact barrier function is required for regulating the timed appearance of the ER protein Ist2 in large G2/M phase buds, as deletion of any barrier component allows premature localization of Ist2 to small S phase buds. Thus, the ER diffusion barrier polarizes the ER by regulating the localization of polarized ER proteins.

219C. Structural and functional analysis of *Saccharomyces cerevisiae* cell surface adhesins. ***N. Wozniak***, *H-U Mösch.* Philipps University Marburg, Department of Genetics, Karl-von-Frisch-Stra rgle 8, 35043 Marbu Germany.
Saccharomyces cerevisiae contains a set of cell wall-associated proteins, the flocculins Flo1, Flo5, Flo9, Flo10 and Flo11, which confer different types of adhesion. The structurally related Flo1, Flo5, Flo9 and Flo10 confer cell-cell adhesion by lectin-like and calcium-dependent binding of mannoproteins on neighboring cells. This heterotypic type of adhesion is known as flocculation and allows *S. cerevisiae* to form protective multicellular flocs. Our previous structural analysis of the adhesion domain (A domain) of Flo5 has revealed that it consists of a conserved PA14 domain, which is also found in

Flo1, Flo9 and Flo10. Furthermore, these PA14-type flocculins carry an additional subdomain that has been suspected to confer ligand binding specificity. In contrast to the PA14-type flocculins, the structurally unrelated Flo11 enables yeast cells to adhere to abiotic surfaces such as agar and plastic by an unknown mechanism. Interestingly, previous studies have shown that Flo10 is also able to confer agar adhesion. In this study we have further characterized the structure and function of the A domains of *S. cerevisiae* PA14-flocculins present in the laboratory strains Sigma1278b and S288c. By using a *FLO11*-based expression system, we demonstrate that with the exception of $Flo9A_{Sigma1278b}$ all PA14-type A domains confer flocculation. We also find, that in addition to Flo11A and Flo10A, the A domain of $Flo1_{Sigma1278b}$ confers weak agar adhesion. To further study the role of the subdomain of PA14-type flocculins, we also analyzed the A domains of Flo5 and Flo10 in more detail, because their subdomains significantly differ in sequence and size. For this purpose, we constructed A domain variants, where the subdomain has been removed ($Flo5A^{G4S}$) or exchanged ($Flo5A^{Flo10SD}$ and $Flo10A^{Flo5SD}$). Our functional analysis reveals that (i) $Flo5A^{G4S}$ is non-functional, (ii) $Flo5A^{Flo10SD}$ no longer confers flocculation, but weak agar adhesion, and (iii) $Flo10A^{Flo5SD}$ confers flocculation, but no agar adhesion. In summary, our data highlight the crucial role of *S. cerevisiae* adhesin A domains in conferring functional specificity and in particular the importance of the subdomain of PA14-type flocculins.

220A. Chromatin regulation of pericentric non-coding RNA in S. cerevisiae affects chromosome stability. ***Julia Allison Gallo***, *Jen Gallagher.* Biology, West Virginia University, Morgantwon, WV.
Centromeres are responsible for the symmetric separation of chromosomes into daughter cells during mitosis. If centromere function is impaired chromosomes can missegregate leading to aneuploidy. Yeast centromeres do not exhibit centromeric silencing compared to organisms with repetitive centromeres but do have specialized chromatin. In other organisms, centromeric transcripts are important for the establishment of the specialized chromatin state at the centromeres which contributes to centromeric silencing and chromosome stability. There are 12 RNAs detected between the centromeres and the nearest protein-coding gene (XU et al. 2009). Six CUTs (cryptic unstable transcripts) were transcribed both toward and away from the centromeres and are only detected in rrp6 mutants. Six SUTs (stable unannotated transcripts) were only transcribed away from the centromeres on the CDEIII element side only and were an average of 400 nucleotides long. Chromatin assembly factors (Cac1 and Asf1) and Sir1 (silent information regulator) stabilize the centromere (Sharp et al., 2003) and regulate transcription centromeric SUTs. Centromeric SUTs are degraded by nonsense mediated decay pathway and other exonucleases. Future studies will address the role of centromeric SUTs in centromere stability.

221B. Dynamic Regulation of the Cnn1-Ndc80 Kinetochore Interaction During Mitosis. *Kriti Shrestha*[1], *Amanda Oldani*[2], *Cinzia Pagliuca*[2], *Peter De Wulf*[2], ***Tony Hazbun***[1]. 1) Dept MCMP, Purdue Univ, West Lafayette, IN; 2) European Institute of Oncology, Department of Experimental Oncology, Milan, 20139, Italy.
The kinetochore is a highly dynamic proteinaceous structure that facilitates the chromosome segregation process. We investigated the interaction of Cnn1 with Ndc80 during the metaphase to anaphase transition. Native PAGE gel and biolayer interferometry binding measurements to identify the minimum-binding domain for Cnn1 that interacts with the Ndc80 complex (Spc24/Spc25 subunits). We demonstrate that $Cnn1^{60-91}$ is sufficient to mediate an interaction with Spc24/Spc25 with a K_D in the low micromolar range but a larger motif, $Cnn1^{25-91}$ increases the affinity by 10 fold. In addition, our two-hybrid and native gel studies demonstrate that the interaction is negatively regulated by phosphorylation at the Mps1 kinase S74 site in Cnn1, whereas other potential phosphorylation sites do not modulate the interaction. Using live cell microscopy, we demonstrate that the presence of the histone fold domain in Cnn1 results in a centromeric localization throughout the cell cycle regardless of the phosphorylation status of S74 site. However, we generated a GFP-Ndc80 binding motif fusion biosensor that enabled the visualization of the cell cycle and phosphorylation-regulated Cnn1-Ndc80 interaction at the metaphase-anaphase transition. Furthermore, our phenotypic assays demonstrate that overexpression of Cnn1 WT and S74A consisting of Ndc80 binding motif result in a synthetic growth defect. Overexpression of the Ndc80 binding motif cannot rescue the *nnf1-17* temperature sensitivity but whereas overexpression of the histone fold domain results in robust rescue. These results suggest that forced Ndc80-Cnn1 interactions can interfere with the function of the essential Ndc80-Mtw1 interaction but that the histone fold domain may have role in compensating for kinetochore deficiencies when the Ndc80-Mtw1 interaction is lacking. In sum, our studies reflect the fine tuned regulation of interactions by phosphorylation and delineate the timing of interaction between Cnn1 and Ndc80 occurring during kinetochore attachment and the chromosome segregation process. We can also infer that the Ndc80 complex must become available for binding to Cnn1 at the metaphase-anaphase transition by an additional regulatory mechanism.

222C. Mechanisms for regulation of kinetochore protein levels in budding yeast. ***Eva Herrero***, *Peter Thorpe.* Stem Cell Biology and Developmental Genetics, National Institute for Medical Research, London, United Kingdom.
Accurate chromosome segregation requires appropriate assembly of kinetochores on centromeres to allow the chromosomes to attach to spindle microtubules. Kinetochores are composed of more that 60 proteins organized into various sub-complexes that are thought to assemble hierarchically on centromeres. Numerous studies in budding yeast

have revealed the stoichiometry of these protein sub-complexes. However, little is know about how these sub-complexes assemble to form the kinetochore and how much flexibility exists in kinetochore composition. To investigate this, we used fluorescence microscopy to quantify the levels of proteins at kinetochore clusters. Mtw1 is an evolutionary conserved member of the MIND complex and is essential in yeast. We have found that Mtw1 levels at the kinetochore correlate with chromosome number. Increasing or reducing MTW1 expression did not lead to changes in the levels of Mtw1p at kinetochore clusters, despite changing cellular Mtw1 protein levels. Our results show that recruitment of Mtw1p to kinetochores is not transcriptionally regulated. This suggests a compensatory mechanism to keep robust Mtw1 levels at the kinetochore. The ubiquitin ligase Psh1p controls the levels of the centromeric-specific H3-variant Cse4p. Consistently we found that Cse4p levels are increased at the kinetochore in *psh1* cells. A second E3 ubiquitin ligase Ubr2p has been found to modulate protein levels of Dsn1, another member of the MIND. We found that the levels of Mtw1 at the kinetochore were significantly elevated in *psh1 ubr2* double mutant, but not in the single *psh1* or *ubr2* strains. We obtained similar results for Dsn1p. We did not find changes in Ndc10p levels in *psh1*, *ubr2*, or *psh1 ubr2* double mutant cells. Taken together, our results indicate that Psh1p and Ubr2p act redundantly to restrict MIND complex protein levels at the kinetochore.

223A. Regulation of centromeric nucleosome localization by the E3 ubiquitin ligase Psh1. ***Erica Marie Hildebrand***[1,2]*, Sue Biggins*[1]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology, University of Washington, Seattle, WA.
The centromeric nucleosome is the epigenetic mark of the centromere, and its localization must be carefully regulated to ensure correct kinetochore assembly and proper chromosome segregation. In budding yeast, the CENP-A^{Cse4} centromeric histone variant is degraded by the E3 ubiquitin ligase Psh1. In cells lacking Psh1, overexpression of CENP-A^{Cse4} is lethal and causes mislocalization of CENP-A^{Cse4} throughout the euchromatin. ChIP-seq of CENP-A^{Cse4} in this strain shows mislocalization specifically to intergenic regions. In addition, CENP-A^{Cse4} mislocalization is enriched in intergenic regions containing promoters, nucleosome depleted regions (NDRs), and H2A.Z^{Htz1}. We are currently investigating the interaction between H2A.Z^{Htz1} and CENP-A^{Cse4} to determine if the presence or absence H2A.Z^{Htz1} nucleosomes at promoters have a causal effect on CENP-A^{Cse4} mislocalization into those regions. In addition, a number of transcripts have decreased levels in the strain with Psh1 deleted and overexpressed CENP-A^{Cse4} compared to the WT strain. These genes with decreased transcripts are also enriched for CENP-A^{Cse4} peaks in the promoter, suggesting that mislocalization of CENP-A^{Cse4} to promoters may have an effect on downstream transcription. This work furthers the understanding of the fundamental question of how the localization of the epigenetic mark of the centromere is regulated, and the functional consequences that occur when this mechanism is perturbed.

224B. Development of an in vitro kinetochore assembly assay to investigate kinetochore function and two alternate assembly pathways. ***Jackie Lang***[1,2]*, Sue Biggins*[1,2]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology, University of Washington, Seattle, WA.
During every cellular division, the cell must accurately segregate its chromosomes in order to avoid aneuploidy, a hallmark of most cancers. Faithful segregation relies on the attachment of the chromosomes to spindle microtubules via the kinetochore, a highly conserved protein complex. The kinetochore is composed of eight major subcomplexes of proteins, which can be broadly categorized into either inner (DNA-associated) or outer (microtubule-associated) kinetochore. While it is agreed that the kinetochore is built from the centromeres of chromosomes outward toward the microtubule attachment sites, the mechanism and regulation of kinetochore assembly is still not well understood. I am developing a cell-free method for kinetochore assembly in order to address numerous questions in the field that cannot be examined in vivo. Centromeric DNA from S. cerevisiae is used as a platform to assemble kinetochores from whole cell extract. I have thus far achieved strong and consistent binding of inner kinetochore components and weak to intermediate binding of several outer kinetochore components. In optimizing this assembly assay, my goal is to achieve levels of outer kinetochore binding that are stoichiometrically similar to what has been measured at kinetochores in vivo using fluorescence microscopy. Ultimately, these assembled particles will be subjected to functional assays, testing their ability to bind microtubules and withstand force. In addition to the canonical mechanism of assembly, recent studies have revealed a group of histone-fold domain containing proteins, CENP-T-W-S-X, that may function as an alternative assembly pathway. CENP-T and the yeast homolog Cnn1 compete with the MIND complex to bind to the same interaction surface of the Ndc80 complex (outer kinetochore). This indicates that there may be distinct, mutually exclusive pathways for linking the centromere to the outer kinetochore. However, it is unknown whether or how Cnn1 interacts with centromeric DNA, how its kinetochore association is regulated, and whether it contributes to de novo kinetochore assembly. Preliminary data suggest that Cnn1 plays a critical role during in vitro kinetochore assembly. Further studies to distinguish its role at the centromere are currently underway.

225C. Molecular Basis of Deleterious Pericentric Recombination during Meiosis. ***Mridula Nambiar****, Gerald Smith.* Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Meiosis generates haploid gametes from diploid cells after two rounds of nuclear division and chromosome segregation. In most species, genetic recombination is required for the proper segregation of chromosomes. Regions in and around the centromeres are repressed for recombination, with a low frequency of crossing over per unit physical distance as compared to the chromosomal arms. Recombination in the pericentric regions can lead to missegregation and thereby congenital defects like Down syndrome and other trisomies. RNAi and heterochromatin mutants are derepressed for recombination between markers across the largest centromere on chromosome 3 in fission yeast [1]. However, the molecular mechanism of repression of meiotic recombination at the centromeres is not completely understood. Using Schizosaccharomyces pombe as a model system, we employ a two-pronged approach towards tackling this problem. In a biochemical approach, we are working towards the purification of native centromeres mediated by the Cre-loxP recombination system, during meiosis. For the first time in any species, this will determine the proteins comprising the kinetochore and pericentric region in a large (~125 kb), complex heterochromatic centromere during meiosis. Further, comparison with a derepressed mutant would help to elucidate the molecular components involved in pericentric repression of recombination. In a genetic approach, we are individually testing the role of cohesin subunits such as Rec11 and Psc3, and other plausible candidates in regulating centromeric recombination. Overall, this study will help elucidate the factors contributing to birth defects in humans and perhaps lead towards ways of preventing such abnormalities. 1. Ellermeier C et al. (2010) Proc Natl Acad Sci USA 107: 8701-5.

226A. Mutations in histones H3 and H4 define nucleosome regions essential for chromosome segregation. *Payel Chaudhuri*, ***Ines Pinto***. Dept Biological Sciences, University of Arkansas, Fayetteville, AR.
Proper attachment of chromosomes to the spindle microtubules during mitosis is essential for accurate chromosome segregation. The centromeric region of chromosomes is known to have a specialized chromatin structure that is essential for normal microtubule attachment; however, the specific chromatin requirements are not well understood. The nucleosome consists of a tetramer of histones H3 and H4 bound by two histone H2A-H2B dimers, which serves as the core octamer to which 146 bp of DNA wrap around twice to form the basic unit of chromatin. The yeast centromere-kinetohore complex is characterized by the presence of a specialized nucleosome that contains Cse4, a histone H3 variant, surrounded by pericentric chromatin, composed of arrays of canonical nucleosomes. We are interested in understanding the role that histones play in establishing the proper centromeric chromatin structure for microtubule attachment and segregation. To this end, we screened a mutant library that carries an amino acid substitution at each position along the length of H3 or H4. The library was screened for defects associated with chromosome segregation, in particular, an increase-in-ploidy phenotype that we had previously identified in H2A mutants. Our results indicate that histones H3 and H4 are permissive to a number of amino acid replacements throughout the length of the protein, however, there are some critical residues that cannot tolerate mutations without causing serious defects in chromosome maintenance. We identified the DNA entry/exit region of the nucleosome to be important for maintaining normal cell morphology and ploidy levels. In particular, residues H3K42, H3G44 and H4K44 are notoriously sensitive to specific amino acid replacements, leading to aneuploidy as well as an increase in ploidy. H3G44 has already been shown by others to be an important residue in chromosome segregation, and a H3G44S mutation can be suppressed by high copy expression of Sgo1 (shugoshin). We tested suppression by Sgo1 overexpression and found that it suppressed all three alleles in the DNA entry/exit region, but not two others, H4L97A and H4G99A, located toward the center of the chromosome. Our results indicate that the nucleosome has compartmentalized functions associated with specific regions, and mutations that alter the structure in those regions can cause specific phenotypes, as the chromosome segregation defects we found on the H3 and H4 mutants. In addition, the H3 and H4 mutants had defective interaction with Sgo1, preventing normal chromosome segregation.

227B. The stromalin conservative domain in the Scc3 subunit of cohesin mediates the interaction with both Mcd1 and the loading complex. *Ola Orgil*[1], *Avi Matityahu*[1], *Thomas Eng*[2], *Vincent Guacci*[2], *Douglas Koshland*[2], ***Itay Onn***[1*]. 1) Faculty of Medicine, Bar-Ilan University, Safed, Israel; 2) Molecular and Cell Biology Dept, University of California, Berkeley, Berkeley, CA USA.
The structural maintenance of chromosome (SMC) complex cohesin is essential for maintaining the fidelity of the genome. Cohesin is also important for gene expression and has a direct effect on human health. The four subunits of cohesin are assembled in a ring. The anti-establishment complex, comprising the cohesin subunit Scc3 and the auxiliary factors Pds5 and Wpl1, regulates the interaction of cohesin with DNA. Scc3 contains a conserved domain of unknown function called the stromalin conservative domain (SCD). Very little is known about the specific role of Scc3 in cohesin activity. In this study we isolated a short, five amino acid insertion mutant located next to the SCD. We show that when overexpressed, the mutant has a dominant negative effect on cell viability, sister chromatid cohesion and cohesin loading onto the chromosomes. Moreover, the mutant protein fails to support cohesin loading as a sole copy. We show that this mutant cannot bind to cohesin through Mcd1, but maintains interactions with Pds5 and Wpl1. Finally, we show that the interaction of cohesin with Scc2 is mediated through multiple interactions. We found that D373 located in the SCD mediates the interaction with Mcd1. Strikingly, other residues in the SCD appear to be involved in chromosome

condensation in a cohesion-independent manner. Our results suggest assigning new functions to Scc3 and reveal its role in maintaining the stability of the genome.

228C. Stability of a Large Amplification in *Saccharomyces cerevisiae*. ***Jamie Pogachar***, *Celia Payen, Maitreya Dunham.* Genome Sciences, University of Washington, Seattle, WA.
Large chromosomal amplifications and deletions, also known as copy number variants (CNVs), are found in many different cancers. However, the inheritance, stability, and fixation of CNVs within a population are widely debated and poorly understood. In an effort to better understand the stability of chromosomal rearrangements, I analyzed the dynamics of a large segmental inverted tandem repeat amplification in the yeast *S. cerevisiae*. This amplification is commonly observed in clones evolved in the laboratory under sulfate-limited continuous culture conditions and contains the sulfate transporter *SUL1*. This amplification is an important adaptive strategy used by the cells to improve their ability to extract the limited supply of sulfate available in the media. We have shown previously that high copy number of this gene confers a competitive fitness advantage over other clones that have only one copy of the gene. We used a GFP marker integrated next to *SUL1* to differentiate green clones, which contain a single copy of the *SUL1* gene, from super green clones that have multiple copies of the gene. I followed the amplicon copy number over time of a super green evolved clone grown in selective sulfate-limited medium versus a different nutrient limitation in which the amplification is neutral. I monitored the population using flow cytometry and quantitative PCR to find clones that have lost the amplification, and to measure the rate at which the amplification is lost. These green clones were isolated using flow sorting and then had their genomes sequenced to look at the scar that was left behind. These experiments tell us how efficiently the cells can revert amplifications to return to a euploid state.

229A. Chromosome Breakage at Potential Fragile Sites in Retrotransposon Overdose Strains. *Cristina M. Lanzillotta*[1], *Samantha Minikel*[1], *Nicholas Monteleone*[1], *Bracha Erlanger*[2], *Sarah J. Wheelan*[2], ***Lisa Z. Scheifele***[1]. 1) Department of Biology, Loyola University Maryland, Baltimore, MD; 2) Department of Oncology, Division of Biostatistics and Bioinformatics, Center for Computational Genomics, Johns Hopkins School of Medicine, Baltimore, MD.
Fragile sites are regions of frequent chromosome breakage. In yeast, a pair of inverted Ty retrotransposons comprise a fragile site (Lemoine 2005), suggesting that all Ty pairs might be destabilizing. We studied chromosome breakage in Retrotransposon Overdose (RO) strains, which contain a greater number of Ty elements and suffer greater levels of genome instability (Scheifele 2009). RO strains were generated through multiple rounds of transposition, producing strains containing between 27 and 37 Ty multimers due to repeated insertions into preferred sequences upstream of tRNA start sites. Genome sequencing of RO strains reveals that these Ty multimers differ in the relative distance and orientation of Ty elements, allowing us to investigate the relative stability of diverse arrangements of Ty pairs. To determine whether Ty pairs would serve as preferential sites of chromosome breakage, we subjected cells to replication stress using a combination of methylmethanesulfonate (MMS) and a temperature-sensitive polymerase- allele (*pol1-17*). These conditions produced chromosome aberrations in 34% of treated cells with cells exhibiting a diverse array of chromosome aberrations. However, several cells had identical electrophoretic karyotypes, suggesting that some sites may break at a higher frequency. While we expected that replication stress would be a common inducer of fragile sites, we compared the spectra of chromosome rearrangement breakpoints in *pol1-17* and *mec1* mutants, which are known to induce different sites of chromosome breakage in cells with wild-type Ty copy number (Cha 2002, Lemoine 2005). While each mutant produced many different chromosome aberrations, they also shared one common chromosome aberration which occurred in 12% and 15% of *mec1* and *pol1-17* mutants respectively. Different inducers of replication stress may therefore induce some breaks at common sites, suggesting that fragile sites vary in their propensity to breakage.

230B. Interface between a two-cohesin complex model of cohesion and DNA replication. *Kevin Tong, Soumya Rudra*, ***Robert V. Skibbens***. Biological Sciences, Lehigh University, Bethlehem, PA.
High fidelity chromosome segregation during mitosis requires that cells identify the products of DNA replication during S-phase and then maintain that identity until anaphase onset. Sister chromatid identity is achieved through cohesin complexes, but the structure through which cohesins perform this task remains enigmatic. In the absence of unambiguous data, a popular model is that some subset of cohesin subunits form a huge ring-like structure that embraces both sister chromatids. This one-ring two-sister chromatid embrace model makes clear predictions - including that premature cohesion loss in mitotic cells must occur through cohesin-DNA dissociation. We used chromatin immunoprecipitation to directly test for cohesin dissociation from cohesin binding sites in mitotic cells inactivated for Pds5 - a key cohesin regulatory protein. The results reveal little if any chromatin dissociation from cohesins, despite a regimen that produces both massive loss of sister chromatid tethering and cell inviability. We further excluded models that cohesion loss in mitotic cells inactivated for Pds5 arise through either cohesin subunit degradation, loss of Hos1-dependent Smc3 deacetylation or Rad61/Wapl-dependent alteration of cohesin dynamics. In combination, these results support an alternate model that cohesin complexes associate with each sister and that sister chromatid cohesion results from cohesin-cohesin interactions that form during DNA replication. Importantly, these results are in strict agreement with new evidence

regarding the cohesion DNA helicase Chl1, yeast homolog of human helicases that when mutated give rise to Fanconi anemia, breast cancer and Warsaw Breakage Syndrome. Chromatin immunoprecipitations revealed that Chl1 promotes Scc2 (and cohesin) deposition onto chromatin specifically during S-phase, consistent with cell cycle mapping studies that both Chl1 expression and chromatin-recruitment peak during S-phase. While Scc2 association onto DNA during G1 is not abrogated in chl1 mutant cells, these cells exhibit significant cohesion defects (Rudra and Skibbens 2013). Thus, Scc2 and cohesin deposition onto DNA during S-phase are required for cohesion. In combination, these results support a model that cohesin loading occurs on each sister chromatid, conceptually linking cohesin deposition, cohesion establishment and chromatin assembly reactions - and that cohesion is maintained through cohesin-cohesin interactions.

231C. Systematic gene over-expression screen for increased mutation rate in *Saccharomyces cerevisiae*. ***Jonathan S Ang***[1], *Supipi Duffy*[1], *Peter C Stirling*[2], *Phil Hieter*[1]. 1) University of British Columbia, Vancouver, Canada; 2) Terry Fox Laboratory, BC Cancer Agency, Vancouver, Canada.
Mutations that cause genome instability are considered important predisposing events that contribute to initiation and progression of cancer. Genome instability can be categorized into defects in genes that cause an increased mutation rate (mutator phenotype) or defects in genes that cause increased aneuploidy rates (chromosome instability (CIN) phenotype). To date, our lab has analyzed loss-of-function and reduction-of-function mutations that cause either CIN or mutator phenotypes. To explore the effects of gene dosage on mutation rate, we systematically tested the consequences of gene over-expression using a forward-mutation screen in the budding yeast. We identified 7 genes that when over-expressed confer a mutator phenotype. Four of the 7 genes were also shown to cause chromosome instability when over-expressed. The identified genes include representative genes from DNA replication and repair pathways, which are known to be important for genome maintenance. Fluctuation analysis revealed that 6 genes confer a strong dosage-mutator phenotype (i.e. greater than 5-fold above wildtype), and several of the genes are associated with cancer. Overexpression of MPH1, the yeast ortholog of Fanconi Anemia gene FANCM, resulted in the strongest mutator phenotype (over 200-fold above wildtype). Mph1 loss of function mutants have previously been shown to cause a mutator phenotype. Further investigation of the phenotypes caused by these dosage-mutator genes will be useful in identifying and characterizing genes whose amplification and/or over-expression result in disease states.

232A. Suppression of the yeast DNA damage response gene *RTT107* by nonphosphorylatable H2A. ***Joshua A.R. Brown***, *Michael S. Kobor*. Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver BC, Canada.
The DNA damage response (DDR) is a highly conserved and vital process for maintaining genome stability and cellular function. Rtt107 is a *Saccharomyces cerevisiae* protein involved in the DDR, and is thought to act as a scaffold, recruiting key DDR proteins to double-strand breaks. *rtt107* strains display DDR-related defects, including hypersensitivity to DNA damage, chromosomal instability, and a delayed restart of replication after DNA damage repair. A pool of Rtt107 acts with the Slx4 endonuclease to prevent Rad9-mediated Rad53 hyperactivation. *rtt107* and *slx4* strains display different phenotypes, such as the spontaneous genome instability of *rtt107*. Physical interactions of Rtt107 also suggest Slx4-independent function in the DDR. Suppressors of *rtt107* and *slx4* DNA damage sensitivity include a nonphosphorylatable *H2A S129A* allele and deletion of the *DOT1* histone methyltransferase. *dot1* suppresses the delayed replication restart of *rtt107*, but does not suppress the genome instability of *rtt107*, and suppression is dependent on functional translesion synthesis (TLS).
We found that suppression of *rtt107* with *H2A S129A* required DNA damage tolerance pathway genes. Triple mutants with deletion of *REV3* or *RAD5*, required for TLS and error-free post-replication repair respectively, lost *rtt107* suppression with *H2A S129A* in multiple agents. These results suggested a mechanism of suppression similar to *dot1*, and we found that this *H2A S129A* allele also rescued the delayed DNA replication restart of *rtt107*. However, as with *dot1*, the *H2A S129A* allele did not suppress the spontaneous genome instability of *rtt107*. These findings suggest that Rtt107 has function in genome stability and damage repair that is partially separate from its role in promoting replication restart.

233B. The State of the Rfa2 N-Terminus Affects Rfa1-Protein Interactions that Map Outside of the Rfa1 N-Terminus. ***Kaitlin M. Dailey***, *Erica N. Mueller, Gunjan Piya, Stuart J. Haring.* Chemistry and Biochemistry, North Dakota State University, Fargo, ND.
The importance of protein interaction networks is significant considering that cellular processes often involve signaling between proteins and/or the temporal and spatial recruitment and displacement of proteins. For example, DNA repair requires the concerted effort of proteins to recognize a DNA lesion, remove that lesion, and process the intermediate(s) formed back into intact double stranded DNA, while also allowing the cell a window of opportunity to do so through an intricate signaling cascade (i.e., checkpoint arrest). Replication Protein A (RPA) is a heterotrimeric complex that is central to DNA replication, repair/recombination, and cell cycle regulation by virtue of its single stranded DNA (ssDNA) binding activity and its ability to interact with components involved before and after the formation of a ssDNA intermediate. It is not clear exactly how RPA interacts with most of these other factors, or how RPA facilitates the activity or loading of

these factors onto DNA following damage. Furthermore, the human Rpa2 subunit is hyper phosphorylated on its N terminus in response to DNA damage, and the role of this post translational modification is also not clear. Purpose: To determine if modification of the Rfa2 N-terminus regulates protein interactions with RFA. Method: Two-hybrid analysis. Results: Interactions between yeast RPA, called Replication Factor A (RFA), and other factors that have been demonstrated to play a role in the DNA damage response are predominantly mediated through the N terminus (DBD F) of Rfa1, whereas factors without an obvious or currently known role in DNA damage repair interact through other regions of Rfa1. Furthermore, a number of these interactions are affected by the formation of DNA damage in the cell; however, these interactions predominantly map outside of DBD F. Finally, a phospho mimetic form of yeast Rfa2 results in disruption of interactions between RFA and proteins that interact with DBD B, consistent with a previous study suggesting that the N terminus of Rpa2, when negatively charged, is altering the structure of DBD B and not DBD F. However, these interactions are only affected in the context of a complete RFA complex (or when at least 3 of the 4 DBDs of Rfa1 are present).

234C. The mismatch repair recognition complex MutS tracks with the replisome. *Joanna E. Haye*, ***Alison E. Gammie***. Dept Molec Biol, Princeton Univ, Princeton, NJ.
During replication, DNA mismatch repair recognizes and repairs mispaired bases that escape the proofreading activity of DNA polymerase. Defects in mismatch repair genes have been linked to compromised genome stability and diseases including cancer. The repair process includes identification of a mismatch in the DNA helix followed by cleavage and excision of the error-containing strand. After the error is removed, a new DNA strand with correct base pairing is synthesized. Our research is directed towards understanding how the genome is efficiently scanned for mismatches and how strand specificity of repair is accomplished once the mismatch is detected. One model addressing both issues is that efficient genome scanning and strand specificity are accomplished by close tracking of the mismatch repair recognition proteins with the replisome during replication. Mismatch scanning can be inhibited by protein blockages such as nucleosomes; however, nucleosomes are effectively cleared by the replisome helicases. In many organisms, mismatch repair is directed to a nicked strand of DNA. In the zone of active replication, nicks are common along the newly synthesized strand. We tested the tracking model using synchronized cells and chromatin immunoprecipitation in combination with custom DNA tiling arrays (ChIP-chip) covering ~70 origins of replication to track movement of the replisome. We first established that the ChIP-chip is an effective method for detecting the leading strand polymerase, Pol, during DNA synthesis. The leading strand polymerase ChIP signal throughout S-phase was detected with the resolution to track origin firing timing and efficiencies as well as replisome progression rates. Importantly, we found that MutS binds origins and spreads to adjacent regions with the leading strand polymerase during replication. Additionally, MutS dynamics are consistent with the origin firing timing and efficiencies observed with the leading strand polymerase data. In summary, we find that the MutS mismatch recognition complex tracks with the replisome during replication. Thus, during replication the MutS complex has proximity to nicks to direct repair to the newly synthesized strand before chromatin reassembles.

235A. Distinct Roles for the Rfa2 N-Terminus in the DNA Damage Response and Adaptation in Saccharomyces cerevisiae. ***Padmaja L Ghospurkar***[1], *Timothy M Wilson*[1], *Amber L Severson*[1], *Sarah J Klein*[2], *Sakina K Khaku*[2], *Andre P Walther*[2], *Stuart J Haring*[1]. 1) Department of Chemistry and Biochemistry, North Dakota State University, fargo, ND; 2) Biological Sciences, Cedar Crest College, Allentown,PA.
In response to DNA damage, two general but fundamental processes occur in the cell: (1) a DNA lesion is recognized and repaired, and (2) concomitantly, the cell halts the cell cycle to provide a window of opportunity for repair to occur. A key factor involved in the DNA damage response is the heterotrimeric protein complex Replication Protein A (RPA), which is not only essential for the repair of damaged DNA, but also is post translationally modified on at least two of the three subunits in response to DNA damage by checkpoint kinases. Of particular interest is the 32 kDa subunit, called Rpa2, which is hyper phosphorylated on its serine/threonine rich N terminus following DNA damage in human cells. This unstructured N terminus is often referred to as the phosphorylation domain (PD) and is conserved amongst eukaryotic Rpa2 subunits, including Rfa2 in Saccharomyces cerevisiae. Purpose: To delineate the importance of this domain in budding yeast. Methods: We utilized an aspartic acid/alanine scanning, genetic interaction, and microscopic approach. Results: We determined that the N terminal putative PD region is important for a proper DNA damage response in yeast, although its phosphorylation is not. We also identified subregions of the Rfa2 N terminus that are important in the DNA damage response. Finally, we showed that a hyper phosphorylation mimetic mutant of the Rfa2 N terminus behaves similarly to another Rfa1 mutant (rfa1 t11) with respect to genetic interactions, DNA damage sensitivity, and adaptation (the ability of a cell to override the G2/M checkpoint despite having unrepaired damage). Our data indicate that post translational modification of the Rfa2 N terminus is not required for cells to deal with repairable DNA damage, but that post translational modification of this domain might be necessary for cells to proceed into M phase in the continued presence of unrepaired DNA lesions as a last resort mechanism for cell survival.

236B. The application of glucose starvation as a selective force for the study of adaptive mutations in yeast. *Maria Hubmann, Petra Dorninger, Agnes Civegna,* ***Erich Heidenreich***. Institute of Cancer Research, Dep. of Medicine I, Medical University of Vienna, Vienna, Austria.
Adaptive mutations are stationary-phase mutations that confer an immediate advantage by enabling a resumption of proliferation. Previous assays for the study of adaptive mutation in *Saccharomyces cerevisiae* mostly relied on rather artificial growth-arresting conditions like starvation for single essential amino acids or nucleotide precursors. Here, in order to mimic natural conditions more closely, we developed an assay in which cell cycle arrest is mediated by glucose limitation. For this purpose, we constructed glucose-auxotrophic strains by manipulating the FBP1 gene. In combination with a non-fermentable carbon source like lactate, we were able to achieve an efficient cell cycle arrest due to a block of gluconeogenesis. There was no residual proliferation after transfer of FBP1-deficient cells to glucose-free medium and subsequent long-term viability was good. Custom-designed frameshift alleles of FBP1 allowed us to screen for adaptive reversions that are caused by compensating frameshifts. Reconstruction assays confirmed that the late-arising revertant colonies obtained with such a setup indeed are the consequence of mutations during cell cycle arrest. We observed that the use of internal frameshifts suffers from some limitations like e.g. low mutation frequency. In contrast, by the insertion of a 5' microsatellite fusion sequence that serves as a frameshift hot spot we could increase the adaptive mutation frequency to an experimentally more convenient value. In summary, this new combination of mutation target and glucose-free selection yields spontaneous replication-independent mutants and enables further studies of the processes behind mutagenesis in resting cells.

237C. Volatility of mutator phenotypes at single cell resolution. ***Alan Herr****, Scott Kennedy, Eric Schultz, Thomas Chappell, Gary Knowels, Brendan Kohrn.* Department of Pathology, University of Washington, Seattle, WA.
Elevated mutation rates caused by mutator phenotypes fuel the evolutionary process of neoplastic transformation. New mutations are assumed to arise at a constant rate in each S-phase. If this assumption is false, an expanding mutator population may contain subclones with widely divergent rates of evolution. Most mutation rate measurements rely on scoring rare mutations in a small target using large populations of cells. The development of Next Generation Sequencing (NGS) technologies permits the entire genome to be used to score mutations. For the first time, it is possible to measure mutation rates of individual cell divisions. Here, we describe single cell resolution mutation rate measurements of mutator *Saccharomyces cerevisiae* cells. Budding yeast divides asymmetrically into mother and daughter cells, which can be easily separated on an agar surface by micromanipulation and then grown into clones. We sequenced the genomes of clones derived from all sequential daughters from mutator mother haploid cells, deficient in Pol proofreading (*pol2-4*) and base-base MMR (*msh6*). We found that the distribution of mutation rates of individual cell cycles did not conform to a single Poisson distribution. Instead, the data best fit a model created with two overlapping Poisson distributions generated with mutation rates that differ by an order of magnitude. Our results demonstrate the feasibility of measuring mutation rates of individual cell divisions and indicate that mutator cells assume distinct mutagenic states.

238A. An experimental system to investigate large-scale CAG/CTG trinucleotide repeat expansions. ***Jane C. Kim****, Samantha T. Harris, Kartik A. Shah, Sergei M. Mirkin.* Biology, Tufts University, Medford, MA.
Expansions of CAG/CTG trinucleotide repeats are the cause of more than a dozen hereditary human diseases, including Huntingtons disease, myotonic dystrophy, and multiple forms of spinocerebellar ataxia. A characteristic feature of these diseases is that they demonstrate genetic anticipation, or an increasing severity of symptoms and earlier age of onset in successive generations, which correlates to increasing length of the expanded allele. Both small- and large-scale expansions are relevant to disease inheritance and severity, yet it is unclear whether these occur by the same or distinct molecular mechanisms. We have developed a selectable budding yeast system to detect and measure the rates of large-scale expansions of CAG/CTG repeats. Specifically, we can observe the addition of more than 100 repeat copies to a starting tract of 140 CAG repeats. In contrast to small-scale instabilities, whose frequency we and others observe in the percentile range, large-scale CAG/CTG repeat expansions are a much more rare event. We present data comparing the rates of large-scale expansions for CAG/CTG repeats versus Friedreichs ataxia GAA/TTC repeats. Our genetic analysis of expansions for both repetitive sequences provides a direct comparison of the instability of different trinucleotide repeats, implying distinct mechanisms governing their expansions. Furthermore, the lack of an effect of the Srs2 helicase on large-scale CAG/CTG expansions, though it has a well-established role in protecting against small-scale expansions, suggests that the mechanisms of small- and large-scale expansions are different.

239B. Stimulation of RNA Polymerase II ubiquitination by yeast RNA 3' processing factors is a conserved DNA damage response in eukaryotes. ***Jason N. Kuehner***[1]*, Hilary Duffy*[1]*, Claire Moore*[2]. 1) Department of Biology, Emmanuel College, Boston, MA; 2) Department of Developmental, Molecular, and Chemical Biology, Tufts University School of Medicine, Boston, MA.
Cells are frequently subjected to stressful conditions, both upon exposure to byproducts of normal metabolism (e.g. reactive oxygen species) and environmental agents (e.g. ultraviolet light, UV). The ability of cells to cope with genotoxic

stress may be a key factor that determines life span, and failure to react to such stress can contribute to chronic diseases such as cancer. To counteract these dangers, eukaryotic cells have evolved a DNA Damage Response (DDR) that promotes recognition and repair of DNA lesions. A critical consequence of the DDR is a transient decrease in the levels of RNA Polymerase II (Pol II) and mRNA, during which time the cell can repair transcribed regions of the genome. UV irradiation of human cells reduces mRNA at least in part by inhibiting RNA 3 processing, and processing factors likewise promote Pol II ubiquitination and degradation. These data indicate that 3 processing factors provide an important link to the DDR, but the scope and mechanism of these interactions are as yet unknown. Our lab has recently shown that UV-induced inhibition of RNA processing is a conserved response in yeast, and UV-type damage induces genome-wide variation in poly(A) sites. We sought to identify additional functional links between 3 processing factors and the DDR and determine the mechanism of coordination using yeast as a model system. We observed that some yeast 3 processing mutants are sensitive to the UV-mimetic drug 4-NQO. Yeast 3 processing mutants exhibit unique genetic interactions with nucleotide excision repair mutants but not with factors in homologous recombination or post-replication repair pathways. Yeast 3 processing mutants also impair the ubiquitination and degradation of Pol II following DNA damage. Overall these results suggest that 3 processing factors promote DNA repair by removing Pol II stalled at UV-type DNA lesions, a functional interaction that is conserved between yeast and human cells. In ongoing experiments we are testing the role of 3 processing factors in recruiting ubiquitination and DNA repair machineries following UV damage.

240C. Two structurally separable functions of Ctp1 in the early steps of DSB repair. ***L. Ma***, *M. Nambiar, N. Milman, GR. Smith.* Basic Science Division, Fred Hutchinson Cancer Research Center, Seattle, WA.
Meiotic programmed DNA double-strand breaks (DSBs) are repaired by homologous recombination. The early steps of the repair include removal of Rec12 (Spo11 homolog)-oligonucleotides from 5 ends of DSBs and resection of naked 5 DNA ends to generate 3 single-stranded DNA which is bond by Rad51 to initiate homologous strand exchange. Ctp1 (Sae2 homolog) is required in both steps. After random mutagenesis of Ctp1, we isolated mutants likely deficient in clipping but proficient in resection (class 1; CPT-sensitive, MMS resistant) or the converse (class 2; CPT-resistant, MMS sensitive). Eight class 1 mutants fall into the C-terminal region (3 mutations in conserved CxxC motif, 1 mutation in conserved RHR motif, 3 mutations with C-terminal truncation). One class 2 mutant falls into the N-terminal region. The viable spore yield of those eight class 1 mutants are 10-104 fold down, but among the viable spores meiotic recombination frequency in was as wild type or only 2-3 fold down. Three tested mutants like ctp1 cant release the Rec12-DNA complexes, but I-SceI induced meiotic recombination is the same as or even higher than that in wild type. We propose that the Ctp1 C-terminal domain functions in Rec12-oligonucleotide release (clipping) and the N-terminal domain functions in DNA end resection.

241A. Cancer-associated exosome mutations cause DNA:RNA hybrids in yeast. ***K. Milbury***[1,2], *Y. Chan*[3], *V. Mathew*[1], *P. Hieter*[2,3,4], *P. Stirling*[1,2,4]. 1) Terry Fox Laborotary, BC Cancer Research Centre, Vancouver, British Columbia, Canada; 2) Genome Science and Technology, University of British Columbia, Vancouver, British Columbia, Canada; 3) Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada; 4) Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.
Chromosome instability (CIN) results in the unequal distribution of DNA between daughter cells. Although the resulting aneuploidy and genetic damage is directly relevant to malignant disease, the mechanisms underlying the CIN phenotype are often uncertain. Defects in RNA processing may contribute to CIN by increasing the pool of unprocessed RNAs in the nucleus, which can then fail to dissociate from, or re-invade, the DNA duplex. The structures formed by these DNA:RNA hybrids, called R-loops, can expose fragile single-stranded DNA to mutagen attack, and impair replication forks, leading to DNA breaks.
We previously found that a temperature-sensitive mutation in yeast (S. cerevisiae), DIS3 (dis3-ts), caused CIN and exhibited increased R-loop formation by chromosome spreads. Dis3 is the catalytic subunit of the exosome, which degrades RNA, and several mutations in the human homologue of this gene are known to be associated with cancers, including a set of mutations in multiple myeloma (MM) that lead to extensive RNA processing defects and growth retardation. Further characterization of endo- and exo-nuclease deficient alleles and of the MM-associated DIS3 mutations in yeast has shown that some also induce DNA:RNA hybrids and increase sensitivity to DNA damaging agents. This set of alleles will allow us to dissect the activities of Dis3 that regulate genome integrity and may contribute to mutational processes in cancer.

242B. Numerous extrachromosomal circular DNA elements in *Saccharomyces cerevisiae*. ***Henrik D. Møller***[1], *Lance Parsons*[2], *David Botstein*[2], *Birgitte Regenberg*[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Copenhagen Ø, Denmark; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, USA.
Extrachromosomal circular DNA (eccDNA) is a somewhat underappreciated type of mutation. Strikingly, many human tumor cells carry genes on eccDNA (double minutes) that promote tumor genesis or confer resistance to anti-cancer treatment. In *Saccharomyces cerevisiae*, several ribosomal, retrotransposon and telomeric genes have been detected on

eccDNA as well as *CUP1-1/CUP1-2*, *GAP1* and *HTA2/HTB2* genes. A central question is how common eccDNA are in eukaryotic cells? We performed a genome-wide screen for eccDNA elements in *S. cerevisiae* that consisted of column purification of eccDNA, degradation of the remaining linear DNA, 29 polymerase amplification, sequencing, mapping and data mining. Screening of $1x10^{10}$ cells from ten populations yielded 1,790 eccDNA elements covering nearly one-fourth of the *S. cerevisiae* genome. We confirmed the existence of a number eccDNA with an average size of 3.5 kb and recorded eccDNA elements as large as 23 kb. Most eccDNA elements were unique to a single population, suggesting they formed as de novo mutations or existed in the common ancestor but were lost by drift in some populations. A small number of eccDNA elements were found repeatedly, containing *HXT6/7*, *ENA1/2/5*, *CUP1-1/-2*, retrotransposons, ribosomal genes and Y telomeric genes. Around one-fifth of all eccDNA elements likely formed by homologous recombination between repetitive elements (e.g. $ENA1/2/5^{circle}$) and almost one-fifth carried known replication origins (e.g. $HXT6/7^{circle}$), supporting a mechanism for long-term persistence of these eccDNA elements in a lineage of cells. Our genome-wide screen for eccDNA indicates that DNA circularization is a common mutational event in *S. cerevisiae* and it is tempting to suggest that eccDNA plays a role in creation of copy number variations and evolution of *S. cerevisiae*. With a detection level of one eccDNA per 2,500 cells, the current method has broad application in screening and research in eukaryotic eccDNA. New studies will provide us with more clues as to how common eccDNA are and potentially elucidate hot spot regions for DNA circularization.

243C. The Role of DNM1 in Mitochondrial Genome Stability in Budding Yeast. ***Christopher T Prevost***, *Deanna Pedeville, Rey A Sia*. Dept. of Biology, The College at Brockport-SUNY, Brockport, NY.
Mitochondria are essential organelles in eukaryotes. Known as the power house of the cell, mitochondria manufacture ATP, which is required for the successful completion of many cellular processes. Mitochondria have individual genomes, separate from the nuclear DNA, which encode for proteins required for respiration. In humans, mutations in the mitochondrial DNA (mtDNA) result in the loss of mitochondrial function which leads to neuromuscular and neurodegenerative disorders. The focus of this study is to determine the role of the nuclear gene *DNM1* in maintaining mtDNA stability in the budding yeast, *Saccharomyces cerevisiae*. Dnm1p protein is a dynamin-related GTPase protein localized to the outer membrane of mitochondria. Mitochondria undergo a constant state of fusion and fission within the cell which allows for mitochondrial segregation during cellular division. Dnm1p is a key regulator of mitochondrial fission. Loss of Dnm1p leads to aberrant mitochondrial structures. The lab is interested in determining whether loss of the *DNM1* gene plays a role in mitochondrial genome stability. We observed in *dnm1* mutants a ~3-fold increase in spontaneous respiration loss which may be a result of altered mtDNA stability. Mitochondrial genome instability can arise via spontaneous point mutations or deletion events. Assays were performed to measure the spontaneous point mutation rate between wild type and *dnm1* mutant strains. Spontaneous point mutation rates were shown to increase in *dnm1* mutants. Strains were constructed to determine the role of *DNM1* in spontaneous direct repeat-mediated deletion (DRMD) events within the mitochondrial genome as well as the nuclear genome. The rate of DRMD events in the mitochondrial genome was increased in the *dnm1* strain compared to that of the wild type. However, there was no change in the rate of nuclear DRMD events for the nuclear genome. The lab has isolated mtDNA from *dnm1* and wild type strains in order to determine structural differences.

244A. Dbf4-dependent kinase regulates both spontaneous and induced mutagenesis by binding to and phosphorylating the Rev7 subunit of DNA polymerase ***Robert A. Sclafani***[1], *Luis Brandão*[1], *Rebecca Ferguson*[1], *Irma Santoro*[3,4], *Sue Jinks-Robertson*[2,4]. 1) Dept Biochem, Molec Gen, Univ Colorado Denver, Aurora, CO; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 3) Department of Biology, 7300 Reinhardt Circle, Reinhardt University, Waleska, GA; 4) Department of Biology, Emory University, Atlanta, GA.
The yeast Dbf4-dependent kinase (DDK, comprised of Dbf4 and Cdc7 subunits) is an essential, conserved Ser/Thr protein kinase that regulates multiple processes in the cell including DNA replication, recombination and induced mutagenesis. Only DDK substrates important for replication and recombination have been identified. Consequently, the mechanism by which DDK regulates mutagenesis is unknown. The yeast *mcm5-bob1* mutation that bypasses DDKs essential role in DNA replication was used here to examine whether loss of DDK affects spontaneous as well as induced mutagenesis. Using the sensitive *lys2A746* frameshift reversion assay, we show DDK is required to generate complex spontaneous mutations, which are a hallmark of the Pol translesion synthesis DNA polymerase. DDK co-immunoprecipitated with the Rev7 regulatory subunit of Pol. Rev7 bound only to the Cdc7 subunit, but interactions with the Rev3 catalytic subunit were not observed. The Rev7 subunit of Pol may be regulated by DDK phosphorylation because immunoprecipitates of yeast Cdc7 and recombinant Xenopus DDK phosphorylated GST-Rev7 in vitro. In addition to promoting Pol-dependent mutagenesis, DDK was also important for generating Pol-independent large deletions that revert the *lys2A746* allele. The decrease in large deletions observed in the absence of DDK likely result from an increase in the rate of replication fork restart after an encounter with spontaneous DNA damage. Finally, synergistic UV sensitivity was observed in *cdc7 pol32* and *cdc7 pol30-K127R,K164R* double mutants, suggesting that DDK may regulate Rev7 protein during post-replication gap filling rather than during polymerase switching by ubiquitinated and sumoylated modified Pol30 (PCNA) and Pol32.

245B. Structure-function analysis of the yeast *ELG1* gene. ***Keren Shemesh***, *Martin Kupiec*. Department of Molecular Microbiology and Biotechnology, Tel Aviv University,Ramat Aviv, Israel.
The RFC complex, composed of a large subunit (Rfc1) and four small subunits (Rfc2-5), loads the processivity clamp PCNA onto DNA during DNA replication. Elg1 is a conserved protein with homology to Rfc1 that forms an RFC-like complex. It was shown that PCNA abnormally accumulates on the chromatin in ELG1 deleted strains, suggesting a role for Elg1 in PCNA unloading. ELG1 is involved in many aspects of genome stability: deletion of the gene causes increased rates of spontaneous recombination, gross chromosomal rearrangements, increased MMS sensitivity and elongated telomeres. In order to find out more about the connection between Elg1s protein structure and its function, specific point mutations were created, and their effect on genome stability was analyzed. So far the most significant results were obtained for mutations in amino acids TT386/7 which are predicted to be at a PCNA interaction site. The two main mutations are Threonine to Alanine (elg1386/7AA) and Threonine to Aspartic acid (elg1386/7DD). Analyzing the phenotypic effects of these mutations will help clarify the connection between Elg1s structure and function and reveal its mechanisms of action.

246C. The dark side of Swi6; genomic screen for mutants hypersensitive to double strand breaks reveals Swi6 indispensability for genome integrity and maintaining of cellular ploidy. *Izabela Brozda*[1], *Paulina Adamus*[1], *Kamil Krol*[1], *Marek Skoneczny*[2], ***Adrianna Skoneczna***[1]. 1) Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland; 2) Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland.
SWI6 was one of the genes indicated in a global screens for *Saccharomyces cerevisiae* mutants sensitive to double strand breaks resulting from zeocin treatment or endonucleases overexpression. Swi6 is multifunctional protein whose engagement in the control of cell cycle progression is well established. Swi6 acts as transcription cofactor in two major complexes MBC and SBC regulating transcription at START thereby governing G1/S transition. Swi6 is also well known for its involvement in response to various stresses, e.g. cell wall stress, oxidative stress, UPR and ER stress. It has been also shown, that Swi6 acts as cellular redox sensor *via* red-ox status of its Cys404 residue. The role played by Swi6 in the cell during stressful condition is assumed to be independent of its involvement in the regulation of cell cycle progression. Our study showed that Swi6 is engaged also in cellular protection against genotoxic stress, especially in the response to DNA double strand breaks. The lack of Swi6, its overexpression, partial dysfunction or even change in *SWI6* gene dosage is manifested by the big changes in yeast cell phenotype. Cells with disturbed proportion of Swi6 molecules per cell or cells containing its altered variant Swi6-C404A: (1) display changes in survival during various genotoxic conditions, (2) lose the ability to repair DNA damage, (3) show abnormalities in DNA content, (4) demonstrate anomalies in nuclei morphology and/or have more than one nucleus per cell. Moreover, our data show that the phenotypes displayed by cells with disturbed Swi6 content or function are also influenced by the cell ploidy. This work was supported by Polish National Science Center grant 2011/03/B/NZ2/00293.

247A. Genomic screen for mutations conferring zeocin hypersensitivity reveals diverse roles of vesicular trafficking paths in genotoxic stress protection and in genome preservation. *Kamil Krol*[1], *Izabela Brozda*[1], *Marek Skoneczny*[2], *Maria Bretner*[3], ***Adrianna Skoneczna***[1]. 1) Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland; 2) Department of Genetics, Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland; 3) Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland.
Cells are constantly exposed to various stresses,which can affect not only current life comfort of the cell, but may have far-reaching consequences. Uncurbed oxidative stress can cause DNA damage, increase in mutation rate and decrease in cell survival. Some substances provoking oxidative damage in the cell act mainly on DNA. One of them is a radiomimetic zeocin, which causes oxidative damage in DNA, inducing predominantly single or double strand breaks. DNA breaks can subsequently lead to chromosomal rearrangements in genomic DNA, especially in diploid cells in which each sequence has its duplicate in the second copy of the genome. In a global screen for mutants sensitive to zeocin, we selected 136 genes whose deletion causes hypersensitivity to this compound of diploid *Saccharomyces cerevisiae* cells. The screen revealed various gene categories: (1) genes connected with stress response, including response to DNA damage stimulus; (2) DNA repair genes, especially connected with homologous recombination and telomere maintenance; (3) genes involved in cell cycle progression, chiefly in the control of cell division checkpoints, both meiotic and mitotic; (5) and genes involved in remodeling of chromatin. But our screen also demonstrated the involvement of vesicular trafficking system in cell protection against DNA damage. The results of our studies implied the importance of vesicular system integrity for various paths of cell protection from zeocin-dependent damage, including the role in detoxification and probably more direct role in genome maintenance processes. As deletion of the genes involved in vesicular trafficking may lead to Rad52 foci accumulation, to changes in total DNA content or even cell ploidy or may preclude proper DNA repair after zeocin treatment, we postulate that functional vesicular transport is crucial for sustaining integral genome. This work was supported by Polish National Science Center grant 2011/03/B/NZ2/00293, and in part by Warsaw University of Technology.

248B. Dynamic behavior of the CIN proteome in response to genotoxic stress. *Veena Mathew[1], Philip Hieter[2],* ***Peter C. Stirling***[1]. 1) Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada; 2) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.
Cells orchestrate a complex and dynamic response to environmental stimuli that damage DNA. Genotoxic stresses can lead to cell death or foster mutations and chromosomal rearrangements that underlie disease states such as cancer. The dynamic nature of the cellular proteome under genotoxic stress is only partially understood. Here we explore the consequences of genotoxic insults on a mini-array of 632 GFP-fusion proteins in the yeast Saccharomyces cerevisiae. This analysis identified 92 localization changes for 41 proteins in response to three genotoxic stresses. Relocalizing proteins fall into a small number of functional groups, including well-characterized components of DNA repair centers and poorly characterized components of other nuclear foci. One previously unrecognized relocalization behaviour is of a core splicing factor of the SF3B complex called Hsh155, which moves to both peri-nuclear and cytoplasmic foci in response to DNA damaging agents. Physical interaction partners of Hsh155 from the SF3B complex do not exhibit the same genotoxin-responsive movement suggesting that the effect does not involve the entire spliceosome. Unbiased genetic interaction profiling of HSH155 temperature-sensitive alleles shows strong connections to several cellular pathways involving essential spliced genes but not to DNA repair. This suggests that the dynamic relocalization behaviour of Hsh155 may reflect protein sequestration rather than a bona fide function in the DNA damage response. Together this functional genomics dataset gives novel insight into the intracellular movements of genome stabilizing proteins and suggests mechanisms that are the subject of ongoing work.

249C. Localization to the nuclear pore complex is required for stabilizing CAG repeats. ***Xiaofeng Su***[1], *Vincent Dion*[2], *Susan M. Gasser*[3], *Catherine H. Freudenreich*[1]. 1) Biology, Tufts University, Medford, MA, USA; 2) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 3) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.
CAG repeats are structure-forming repetitive DNA sequences which can interfere with DNA replication and DNA repair. CAG repeats are prone to breakage, and repair of the breaks can cause repeat expansions or contractions. Expansion beyond a threshold of ~35 CAG repeats is the cause of more than 14 human diseases including Huntingtons disease and myotonic dystrophy. Studies in yeast have shown that irreparable DNA double strand breaks or collapsed replication forks localize to the nuclear periphery to promote proper repair. We tagged an expanded CAG repeat locus with GFP (green fluorescent protein) on a yeast chromosome, and visualized the localization of the repeats in the nucleus by using fluorescent microscopy. Our data showed that expanded CAG-70 and -130 repeats are more likely than a control to localize to the nuclear periphery during S-phase, in a tract length dependent manner. Through a mobility analysis, we also showed that the mobility of expanded CAG repeats decreases due to this localization. By chromatin immunoprecipitation (ChIP), we found that expanded CAG repeats interact with the Nup84 component of the nuclear pore complex. Moreover, Nup84 and its associated SUMO targeting ubiquitin ligase (STUbL), Slx5/Slx8, are important for preventing CAG repeat instability and fragility. Our results indicate that lesions associated with structure-forming repeats trigger a repair or fork restart event that occurs at the nuclear periphery, and that this event is crucial for the maintenance of genome stability.
This study was funded by Tufts University.

250A. Interchangeable Parts: Determining Rpa2 N-Terminal and Loop 3-4 Function through the Use of Human/Yeast Rpa2 Hybrid Proteins. ***Timothy M Wilson****, Kaitlin M Dailey, Anna Herauf, Jenna Steffes, Erica N Mueller, Padmaja L Ghospurkar, Stuart Haring.* Chemistry and Biochemistry, North Dakota State University, Fargo, ND.
It is of critical importance for the proper growth of a cell that the integrity of the genome is maintained with high fidelity. This occurs through accurate DNA replication and through recognition and repair of DNA lesions before they become permanent mutations. A key factor involved in both DNA replication and the DNA damage response is the heterotrimeric single-stranded DNA (ssDNA) binding complex called Replication Protein A (RPA). Although the RPA complex appears to be structurally conserved throughout eukaryotes, the primary amino acid sequence of each subunit can vary greatly. The interchangeability of orthologous RPA subunits and regions has been examined to some extent; however, because RPA is a complex, it is not surprising that previous studies have had limited success when exchanging only one of the subunits. Purpose: To determine if human RPA, or at least regions of human RPA important for its regulation, can function properly in yeast cells. Methods: We used domain swapping, alanine/aspartic acid scanning, phenotypic, and biochemical analysis of human/yeast Rpa2 hybrids. Results: We demonstrate that exchanging one RFA subunit for the corresponding RPA subunit cannot function due to lack of appropriate interactions among the RFA/RPA subunits. Also, the exchange of the entire RFA complex in yeast with the entire human RPA complex results in cells inviability, indicating that differences in primary amino acid sequence are enough to prevent proper function of the human complex in yeast. However, substitution of yeast Rfa2 with domains/regions important for human Rpa2 function (i.e., the phosphorylation domain and the loop 3-4 region; PD and L34, respectively) result in viable cells, and analogous mutants demonstrate similar phenotypes, especially when examining phospho-mutants and their response to DNA damage. Finally, the human Rpa2 PD attached to Rfa2 can be phosphorylated in a similar manner to human Rpa2, indicating that conserved yeast kinases recognize the human

domain in yeast. The implication of these studies is that yeast has potential as a model system for studying human RPA directly, provided the entire human complex is present.

251B. The role of nucleoporins, specifically Nup2, during meiosis in budding yeast. ***Daniel Chu***, *Sean Burgess.* MCB, UC Davis, Davis, CA.
Meiosis is a specialized form of cell division during which diploid organisms form haploid gametes or spores. To halve the number of chromosomes, cells undergo a single round of DNA replication followed by two rounds of DNA segregation. During the first round of segregation, MI, homologs each containing 2 sister chromatids pair and segregate into each daughter cell. During meiotic prophase programmed double strand breaks (DSBs) are catalyzed by Spo11. These DSBs are required to physically link the homologs in the form of crossovers (COs) to generate the tension ensuring the homologs segregate away from each other. Failure to properly segregate the homologs results in nondisjunction leading to aneuploidy, a leading cause of human birth defects. The nuclear pore complex (NPC), made up of nucleoporins (nups), is a large macromolecular ring structure which sits in the nuclear envelope (NE). Its primary goal is to act as a semi-permeable membrane to allow transport of cargo across the NE. The NPC also functions to regulate the chromatin architecture through DNA repair, chromatin modifications, and expression levels. Since the role of the NPC has been largely unexplored during meiosis, we made knockouts of several nups in Saccharomyces cerevisiae. Interestingly we found that loss of nup2 and nup60 suppresses the mitotic temperature sensitivity of the SK1 yeast strain at 37C. Loss of several of the nups results in defects in spore viability and meiotic progression. Loss of nup2 combined with loss of ndj1, required for tethering the telomeres to the NE, results in a complete arrest before MI which is surprising since loss of either protein produces modest meiotic defects. Inability to repair to DSBs is not the cause of the arrest since DSBs appear and then disappear and COs form albeit severely delayed. Additionally loss of spo11 partially suppresses the arrest since nup2 ndj1 spo11 cells progresses past the first meiotic division but shortly after MI the nuclei to fragment with DAPI staining bodies appearing throughout the cell. It is unclear if the meiotic defect occurs at MII or is only realized at MII. The defects caused by nup2 are not due to transport as a 125AA (~17% full length) truncated version of Nup2 lacking all transport domains is fully competent for meiosis and does not synthetically arrest with ndj1. Boundary activity, which regulates the spread of heterochromatin, has also been attributed to Nup2 but does not play a role in meiosis since the loss of nup60 or htz1, each of which are required for boundary activity, do not phenocopy the loss of nup2. A forward genetics screen for suppressors of the nup2 meiotic arrests has identified several candidates involved with RNA processing.

252C. Tethering of Meiotic Recombination Hotspots. ***Kyle R Fowler***, *Gerry R Smith.* Fred Hutchinson Cancer Research Center, Seattle, WA.
During meiosis homologous chromosome pairs must segregate to opposite poles during the first meiotic division to form viable gametes (spores in fungi). In most organisms this is facilitated by recombination between homologs initiated by developmentally programmed DNA double-strand breaks (DSBs). These DSBs are generated by the conserved topoisomerase-like protein Spo11 (Rec12 in the fission yeast Schizosaccharomyces pombe) and in some organisms are concentrated in hotspots, regions of the genome where breakage occurs much more frequently. However, the regulation of these potentially dangerous lesions, both in their formation and resolution, is not well understood. We recently found in S. pombe that hotspot DSBs are limiting, with adjacent hotspots competing for breakage. This DSB competition extends ~150 kb and indicates that neighboring hotspots communicate, perhaps physically. We also found that linear element (LinE) components Rec25, Rec27, and Mug20 bind chromatin with high specificity at hotspots and are required for practically all hotspot DSBs; thus, these proteins are genome-wide determinants of meiotic DSB hotspots, the first such described. We postulate that at DSB hotspots all four chromatids are bound by these components which then tether neighboring hotspots and permit a single DSB to be made within each cluster. As predicted, we find by a type of chromosome conformation capture analysis that DNA in a Rec27 immunoprecipitate is enriched for interactions with hotspots over ~150 kb. This spatial enrichment depends on the meiosis-specific cohesin Rec8, as does full LinE assembly, but is independent of DSB formation. Our results are consistent with DSB hotspots being in close proximity and this organization regulating DSB formation. In other species with break interference, a single DSB among tethered hotspots may also contribute to crossover interference.

253A. Activation of Holliday junction resolution via phosphorylation of Eme1 in meiosis. ***Randy W. Hyppa***[1], *Pierre-Marie Dehé*[2], *Pierre-Henri Gaillard*[2], *Gerald R. Smith*[1]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Cancer Research Center of Marseille, Marseille, France.
The programmed formation and repair of DNA double-strand breaks (DSBs) during meiosis generates crossovers important for chromosome segregation and the genetic diversity of gametes. After a DSB is made on a chromosome, the intact homolog is invaded by a resected DSB end and used as a template to repair the broken DNA. This creates an intertwined four-stranded DNA intermediate called a Holliday junction (HJ) that must be enzymatically resolved back into duplex DNA. Failure to resolve HJs results in cell death. In the fission yeast *Schizosaccharomyces pombe*, the protein heterodimer Mus81-Eme1 is responsible for the resolution of HJs; in cells lacking either subunit, HJs (predominantly

single) accumulate, no crossovers are formed, and very few viable spores are formed. During mitotic growth Eme1 activity is stimulated by phosphorylation induced by DNA damage [1]. Here, we examined Eme1 during meiosis. We found that Eme1 is heavily phosphorylated during meiosis, and this phosphorylation is dependent on the meiotic transcription factor Mei4. Phosphorylation sites identified after mitotic DNA damage were also seen in meiosis, but there were many additional meiosis-specific sites. The mitotic checkpoint kinases responsible for damage-induced phosphorylation had no detectable role in meiosis. These data suggest a meiosis-specific regulation of Mus81-Eme1 by phosphorylation of the Eme1 protein during meiotic recombination, when large numbers of HJs (~60/cell) must be resolved.
1. Dehé, P.M., *et al.*, *Nat Struct Mol Biol* (2013) **20**:598-603.

254B. A potential novel mechanism for DNA double-strand break repair pathway choice. *Tatsuya Ii, Hiromi Ando, Melanie Alvarado, **Miki Ii**.* Biological Sciences, University of Alaska Anchorage, Anchorage, AK.
DNA double-strand breaks (DSBs) are critical for cell survival and genome integrity. Mutations of DSB repair (DSBR) genes are known to cause predisposition to cancers and accelerated aging in humans. There are two major pathways for DSBR: Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR). NHEJ directly ligates the broken ends, whereas HR requires an intact template for error-free repair. Due to the difference of end products, pathway choice is important in DSBR. During the course of study using *Saccharomyces cerevisiae*, we have characterized 3 parallel pathways of HR. The 3 pathways are individually regulated by Sgs1-Top3-Rmi1, Mus81-Mms4 and RNase H2. We reported that RNase H2 appears to function both upstream and downstream of HR, as does Sgs1-Top3-Rmi1, based on systematic genetic analyses. In addition to its function downstream of HR by dissolving dHJs, Sgs1-Top3-Rmi1 (STR complex) acts in resection at DSBs to generate ssDNA tails. This upstream function of STR complex lies in parallel to Exo1 and is needed prior to strand invasion mediated by Rad51. Importantly, we found that *sgs1* and RNase H2 mutants display similar phenotype in regard to suppression of DNA damage sensitivities of *rad54*, *rad55* and *rad57*. This strongly suggests that RNase H2 functions upstream of HR. Using the yeast strain that cannot undergo HR, we found that RNase H2 deletion increases survival rate in the presence of site-specific HO enconuclease *in vivo*. Since NHEJ is the major system to repair DSBs in the absence of HR, this result suggests that loss of RNase H2 increased frequency of NHEJ in the cell to repair DSBs. We also found that this phenotype of RNase H2 mutants was similar to that of *sae2* and *mre11*. Moreover, the effect of *SAE2* deletion in the *rnh201* mutant was additive in regard to survival rate, as well as the effect of *MRE11* deletion in the *rnh201* mutant. This result strongly suggests that RNase H2 functions in parallel to Mre11-Rad50-Xrs2 (MRX) complex to suppress NHEJ in the early step of DSBR. Interestingly, ribonuclease activity if RNase H2 is not required for suppression of NHEJ. Moreover, our ChIP assay detected binding between RNase H2 subunits and HO-induced DSBs in a similar manner to Rad51. Furthermore, we found that expression of RNase H2 is cell cycle and *YKU70* dependent. Taken together, our data suggests that RNase H2 may be involved in pathway choice for DSBR.

255C. Highly specific contractions of a single CAG/CTG trinucleotide repeat by TALEN, in yeast. ***Guy-Franck Richard**, David Viterbo, Varun Khanna, Valentine Mosbach, Bernard Dujon.* Institut Pasteur, Paris, France.
Trinucleotide repeat expansions are responsible for more than two dozens severe neurological disorders in humans, including Huntington disease, type 1 myotonic dystrophy, Fragile X syndrome and Friedreich's ataxia. A double-strand break between two short CAG/CTG trinucleotide repeats was formerly shown to induce a high frequency of repeat contractions in yeast. Now, using a dedicated TALEN, we show that induction of a double-strand break into a CAG/CTG trinucleotide repeat in heterozygous yeast diploid cells results in gene conversion of the repeat tract with near 100% efficacy, deleting the repeat tract. Induction of the same TALEN in homozygous yeast diploids leads to contractions of both repeats to a final length of 3-13 triplets, with 100% efficacy, in cells that survived the two double-strand breaks. Whole-genome sequencing of surviving yeast cells shows that the TALEN does not increase mutation rate. None of the other 150 CAG/CTG repeats of the yeast genome showed any length alteration or mutation. No large genomic rearrangement such as aneuploidy, segmental duplication or translocation was detected by pulse-field gel electrophoresis. It is the first demonstration that induction of a TALEN in an eukaryotic cell leads to shortening of trinucleotide repeat tracts to lengths below pathological thresholds in humans, with 100% efficacy and very high specificity. Trinucleotide repeat tract lengths were sequenced after TALEN induction, and show both homozygous and heterozygous tract lengths, suggesting that both intra-molecular and inter-molecular double-strand break repair mechanisms are involved in repairing TALEN breaks. These mechanisms will be discussed.

256A. Redefining the *M26* hotspot. ***Walter Steiner**, Chelsea Recor, Bethany Zakrzewski.* Dept Biol, Niagara Univ, Lewiston, NY.
The *ade6-M26* hotspot is a well-characterized hotspot in the fission yeast *Schizosaccharomyces pombe*. This hotspot requires a seven nucleotide sequence, ATGACGT, for activity, which serves as a binding site for the Atf1-Pcr1 transcription factor. Deletion of either the *atf1* or *pcr1* genes eliminates activity of the hotspot. The *M26* hotspot is active in meiosis, but not mitosis. In addition, it is active on a chromosome, but not on a plasmid containing a 3 kilobase clone of the *ade6-M26* allele. Subsequent studies of the *M26* hotspot showed that a larger 10 bp version of the *M26* motif creates a

hotspot that is approximately 3-fold more active than the seven bp sequence. Surprisingly, this more active form of *M26* shows significant hotspot activity on a plasmid. And hotspot activity increases when two or three copies of the sequence are placed in tandem, resulting in greater than 20% of cells recombining to become *ade6*$^{+}$. Virtually all of these recombinants carry the *ade6*$^{+}$ allele on the plasmid, which is characteristic of hotspots as recipients of genetic information. This plasmid-borne hotspot is strongly dependent on Atf1, but not Pcr1 or Rec12. The high frequency of Ade^{+} recombinants are present following vegetative growth of cultures, and no further increase is observed following meiosis. Thus, on a plasmid *M26* acts as a mitotic, rather than a meiotic, hotspot.

257B. The *Schizosaccharomyces pombe* mitochondrial recombination junction- resolving enzyme is functionally homologous to Cce1p of *Saccharomyces cerevisiae*. ***Stephan Zweifel***, *Cody Finke, Kristin Andrykovich, Margaret Alexander, Jean Bower*. Department of Biology, Carleton College, Northfield, MN.
The mitochondrial DNA (mtDNA) in yeast cells undergoes homologous recombination to produce four-way DNA junctions that must be resolved in order to facilitate chromosome separation during organelle replication. Encoded by nuclear genes, the proteins Cce1p in *Saccharomyces cerevisiae* and Ydc2p in *Schizosaccharomyces pombe* are imported into their respective mitochondria where the enzyme cleaves the mtDNA recombination junctions. The absence of the mtDNA resolvase results in an aggregation of linked mtDNA molecules, and an elevated loss of the mitochondrial genome during mitotic division. To explore the idea that Cce1p and Ydc2p are homologs whose function is to resolve the recombination intermediates, we introduced the *YDC2* gene on a plasmid vector into *S. cerevisiae*. Using a GFP-YDC2 fusion, we show that the *S. pombe* resolvase retains its mitochondrial targeting ability when expressed in *S. cerevisiae*. Furthermore, DAPI staining of cells, and gel electrophoresis of isolated mtDNA, indicate that the expression of *YDC2* in an *S. cerevisiae* strain containing the *cce1* null allele significantly reduces the aggregation of mtDNA. The direct impact on mitochondrial genome transmission by this cross-species expression was demonstrated by the ability of Ydc2p to reduce the loss of mtDNA during mitotic divisions in a *cce1 S. cerevisiae* strain. Finally, we show that the overexpression of either *CCE1* or *YDC2* results in the increased mitotic loss of mtDNA in a wild type strain of *S. cerevisiae*. This study suggests that Cce1p and Ydc2p are evolutionarily conserved enzymes that resolve the branched networks of mtDNA resulting from homologous recombination between mtDNA genomes.

258C. Autonomously replicating sequences from *Kluyveromyces marxianus* apparently without canonical consensus. ***Babiker M.A. Abdel-Banat***[1,2], *Hisashi Hoshida*[2], *Rinji Akada*[2]. 1) Dept. of Crop Protection, University of Khartoum, Khartoum North, Sudan; 2) Dept. of Applied Molecular Bioscience, Yamaguchi University Graduate School of Medicine, Ube, Japan.
The structural domains of eukaryotic replication origins are best understood in the budding yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, where some autonomously replicating sequences (ARS) confer origin activity. Although structurally diverse, the best characterized ARS elements of *S. cerevisiae* and *K. lactis* maintain a basic structure composed of three domains, A, B and C. Domain A is comprised of an ARS consensus sequence (ACS), while the B domain has the DNA unwinding element and the C domain is important for DNA-protein interactions. The ACS is commonly composed of 11 bp, 5-(A/T)AAA(C/T)ATAAA(A/T)-3, in *S. cerevisiae* and *K. lactis*. This core sequence is essential for ARS activity exemplified by the fact that the mutations or small deletions within ACS completely abolish its activity. In this study, we have identified more than 28 ARS from a genomic library of the yeast *K. marxianus* and validated their functions. Sequence analysis of these ARS revealed no consensus sequences similar to those of *S. cerevisiae* and *K. lactis*. The yeast *K. marxianus* ARS (KmARS) are highly active but they are divergent and without common consensus. We have carried out mutations, deletions, and bases substitutions within the sequences of some of the short active ARS to identify the core sequence(s) essential for KmARS activity. Our results showed that the 11 bp ACS that commonly found in *S. cerevisiae* and *K. lactis* was not conserved in *K. marxianus*. Moreover, different KmARS sequences are exchangeable, for instance, the short non functional portion of KmARS7 becomes highly active in combination with the non functional portions of KmARS11, KmARS16, KmARS18, KmARS22, KmARS36, or KmARS51. We have also specifically identified the essential nucleotides within the short active KmARS using single nucleotide mutations. It is noteworthy that, a similar sequence to those found in the 60 bp active KmARS7 was also recognized in the 60 bp ARS of the yeast *Candida guilliermonddii*. Our deletions results revealed that the shortest active KmARS is only 21 bp. We can conclude that the identified KmARS are unique compared to other yeasts and they do not share common ACS though they are exchangeable. This may explain the observation of an extensive chromosomal rearrangement in *K. marxianus*.

259A. Tight coevolution of proliferating cell nuclear antigen (PCNA)-partner interaction networks in fungi leads to interspecies network incompatibility. ***Amir Aharoni***[1,2], *Lyad Zamir*[4], *Inga Sandler*[1], *Eitan Rubin*[3]. 1) Department of Life Sciences, Ben-Gurion University, Beer-Sheva, Israel; 2) National Institute for Biotechnology in the Negev, Ben-Gurion University, Beer-Sheva, Israel; 3) Department of Microbiology and Immunology, Ben-Gurion University, Beer-Sheva, Israel; 4) Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel.

The structure and connectivity of protein-protein interaction (PPI) networks are maintained throughout evolution by coordinated changes (coevolution) of network proteins. Despite extensive research,relatively little is known regarding the molecular basis and functional implications of the coevolution of PPI networks. Here, we used the yeast proliferating cell nuclear antigen (PCNA), a hub protein that mediates DNA replication and repair in eukaryotes, as a model system to study the coevolution of PPI networks in fungi. Using a combined bioinformatics and experimental approach, we discovered that PCNA-partner interactions tightly coevolved in fungal species, leading to specific modes of recognition. We found that fungal proliferating cell nuclear antigen-partner interaction networks diverged into two distinct groups as a result of such coevolution and that hybrid networks of these groups are functionally noncompatible in Saccharomyces cerevisiae. Our results indicate that the coevolution of PPI networks can form functional barriers between fungal species, and thus can promote and fix speciation.

260B. Characterizing the mechanism of variation in genomic uracil content . ***Debra Sue Bryan***, *Kerri York, Jay Hesselberth.* Biochemistry and Molecular Genetics, Univ Colorado School Medicine, Aurora, CO.
We developed a method to identify the positions of uracil in genomic DNA with single-base precision. Using in vitro base excision combined with next-generation DNA sequencing, we determined uracil content in strains of E. coli and S. cerevisiae that accumulate uracil in their genomes by the combined inactivation of dUTPase (S.cerevisiae DUT1) and uracil-DNA glycosylase (S.cerevisiae UNG1). In our initial application of this method, we discovered a positional bias in uracil content: the earliest replicating regions exhibit a striking reduction in uracil content. In addition, late replicating regions in yeast also show a depletion of uracil. We are currently testing a model of uracil variation in which nucleotide pool size and composition govern genomic uracil incorporation. We altered the activity of nucleotide biosynthesis enzymes thymidylate synthase (TS), dCMP deaminase, and ribonucleotide reductase (RNR) to determine whether uracil content is altered in the genome. Inhibition of TS with 5-fluoro uracil in ung1 yeast recreates the pattern of uracil depletion at origins, suggesting that overall reduction of dTTP does not increase uracil incorporation in early S phase, and temporal regulation of nucleotide biosynthesis is critical for determining genomic uracil content. Deletion of dCMP deaminase, which synthesizes dTTP continuously during the cell cycle, causes uracil incorporation sooner after origin firing, suggesting that the ratio of U:T determines when uracil is incorporated. Deletion of SML1 increases RNR activity (required to synthesize all dNTPs) throughout the cell cycle and abolishes uracil depletion at replication origins, suggesting that during normal RNR regulation, uracil is excluded from origins because of its low availability during G1. Increasing the dUTP pool in this discrete window of time allows for incorporation at replication origins. Together, these data suggest that uracil availability governs its incorporation into the genome, and a higher U:T ratio at a given time increases uracil incorporation at that time. Because a major consequence of antifolate chemotherapy is an increase of uracil content in DNA, identification of factors that influence genomic uracil content may have implications for understanding uracil-mediated chromosomal instability in humans.

261C. Genetic analyses of the Cdc24-Cds1 interaction in *Schizosaccharomyces pombe*. ***G. M. Guerrero***, *M. Nguyen, E. S. Sison, S. G. Pasion.* Biology, San Francisco State University, San Francisco, CA.
Cdc24 in *Schizosaccharomyces pombe* is an essential lagging strand synthesis factor. Cdc24 mutants (*cdc24-M81*, *-M38*, and *-G1*) exhibit a *cdc* phenotype at restrictive temperatures and only the truncation mutants (*M38* and *G1*) exhibit chromosome breakage. This loss of genome integrity suggests that Cdc24 interacts with components of the DNA damage response, namely the S-phase checkpoint $Cds1^{Chk2}$. Our goal is to understand mechanisms of genome stability since these pathways inhibit tumorigenesis in humans. The Cdc24 protein contains a possible fork-head associated (FHA) binding motif that could bind with the $Cds1^{Chk2}$ FHA domain so we hypothesized that the proteins physically interact. We used a genetic approach to test this possibility, first by overexpressing kinase-active (*cds1*$^{+}$) and kinase-inactive (*cds1-kd*) $Cds1^{Chk2}$ alleles in *cdc24*. We found that overexpressed $Cds1^{Chk2}$ (*cds1*$^{+}$ and *cds1-kd*) was toxic in *cdc24* truncation mutants. This result suggests that the proposed Cdc24-$Cds1^{Chk2}$ interaction does not require the kinase activity of $Cds1^{Chk2}$ and that the interaction is likely direct. We next investigated the cause of the toxicity by overexpressing $Cds1^{Chk2}$ in *cdc24* double mutants. Among the mutants we transformed were the *cdc24rad3* and *cdc24mus81* double mutants. $Rad3^{ATR}$ and Mus81 are upstream and downstream effectors of $Cds1^{Chk2}$ respectively. $Rad3^{ATR}$ can initiate a DNA damage response through $Cds1^{Chk2}$ or through the Chk1 G2/M checkpoint and Mus81 is a Holliday-junction resolvase-endonuclease negatively regulated by $Cds1^{Chk2}$. Mus81 causes genome fragmentation in *cdc24* (unpublished results) so a possibility existed that the toxicity is Mus81-dependent. We found that $Cds1^{Chk2}$ was toxic in truncation mutants of *cdc24* in a *mus81*, but not in a *rad3* background. We conclude that the toxicity in *cdc24* requires the activation of a Rad3-dependent DNA damage pathway, and the toxicity is not dependent on Mus81. Additional studies will assess the requirement of other checkpoint effectors leading to $Cds1^{Chk2}$ mediated toxicity in *cdc24*.

262A. The human Meier-Gorlin Syndrome mutation in *ORC4* reduces replication initiation and rDNA copy number in Saccharomyces cerevisiae. ***Joseph Carlo Sanchez***, *M.K. Raghuraman, Bonny Brewer.* Genome Sciences, University of Washington, Seattle, WA.

Meier-Gorlin Syndrome (MGS) is a rare form of primordial dwarfism, whose features include short stature, small ears, and missing kneecaps. Recently, mutations in the Pre-Replication Complex (*ORC1*, *ORC4*, *ORC6*, *CDC6*, and *CDT1*), which acts at replication origins to initiate DNA synthesis, have been shown to be responsible for MGS (Guernsey et al. *Nat Genet* 2011 and Bicknell et al. *Nat Genet* 2011). How perturbing origin initiation might lead to the phenotypes observed in MGS is currently unclear. Tools for studying replication initiation dynamics in humans are limited. Fortunately, there are several well-established tools to study this process in yeast. Because the MGS mutations in *ORC4* and *ORC6* are in residues conserved between humans and yeast, we have replaced the wild type yeast genes with alleles that contain the human mutations. We find that the *orc4*^{MGS} allele causes slow growth and confers temperature sensitivity and hydroxyurea sensitivity to yeast cells. Under permissive growth conditions the mutation results in an accumulation of cells in G1 and a reduction of origin firing at several origins that we have tested by 2-D gel electrophoresis. We have also discovered that the size of chromosome XII is drastically reduced as a consequence of reduction in the copy number of the rDNA repeats from ~100 to fewer than 15. We are currently testing whether this reduction in rDNA repeats affects ribosome biogenesis and whether the growth defect is due primarily to a chromosome replication problem or to a protein synthesis problem. We are also currently phenotype testing the *orc6*^{MGS} mutant in yeast. In addition to characterizing how the MGS mutations affect origin initiation, this study provides an opportunity to understand how the DNA replication machinery may be regulated by the rDNA locus and how reducing the number of repeats in the rDNA locus affects ribosome biogenesis. Because the origin recognition and pre-RC complexes are highly conserved between yeast and humans, we anticipate studying the consequences of mutations in yeast will provide insight to how they contribute to the human phenotypes in Meier-Gorlin Syndrome.

263B. Ku primarily impacts telomere length in *Saccharomyces cerevisiae* via Est1 recruitment to the telomere. ***Laramie Lemon***[1]*, Jaime Williams*[1]*, Faissal Ouenzar*[2]*, Pascal Chartrand*[2]*, Alison Bertuch*[1]. 1) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 2) Department of Biochemistry, Université de Montréal, Quebec, Canada.
Telomeres are nucleoprotein complexes that cap the ends of linear eukaryotic chromosomes, protecting them from degradation, recombination and fusion, thus contributing to genome stability. Telomeres are maintained by telomerase which, in *S. cerevisiae*, consists of an RNA component, TLC1, the reverse transcriptase Est2, and Est1 and Est3, which are required for telomerase activity *in vivo*, but not *in vitro*. Est1 recruits telomerase to the telomere during late S phase by interacting with Cdc13, a single-stranded telomeric DNA binding protein. The evolutionarily conserved Ku heterodimer also contributes to telomere length maintenance by associating with TLC1 at a unique stem loop structure. In contrast to strains lacking telomerase components, telomeres are short yet stable and cells do not senesce in the absence of Ku or Ku:TLC1 interaction. Strains lacking Ku or Ku:TLC1 binding, such as *yku80-135i*, fail to localize TLC1 to the nucleus and experience reduced levels of Est2 and Est1, possibly secondary to less Est2, at the telomere. Thus, the primary role of Ku in telomere elongation has yet to be fully elucidated. Our data support a model in which Kus major function in telomere length regulation is via effects on Est1. Unlike Est2, when we tethered Est1 to the telomere via Cdc13, Kus role in telomere elongation was bypassed, with telomeres progressively elongating in *yku80* or *yku80-135i* strains comparable to wild type. Furthermore, we found Ku in a complex with Est1; this interaction was dependent on Kus ability to bind TLC1, as the interaction was abolished in a *yku80-135i* strain or upon treatment with RNase A. Although the presence of both Est1 and Est2 at the telomere is mutually dependent, we found tethering Est2 to the telomere via Cdc13 did not rescue Est1 levels at telomeres in a *yku80* or *yku80-135i* strain, despite partial restoration of TLC1 in the nucleus. These data suggests that Kus primary role in telomere elongation lies in its interaction with Est1, and not with Est2 recruitment or TLC1 nuclear localization. The mechanism behind the role of Ku in Est1 recruitment to the telomere is currently under investigation.

264C. *S. cerevisiae* telomerase RNA, TLC1: two new essential functional features in addition to flexible scaffolding. *K.J. Lebo, M.A. Mefford, R.O. Niederer,* ***D.C. Zappulla***. Department of Biology, Johns Hopkins University, Baltimore, MD.
The telomerase enzyme maintains telomeres in many eukaryotes, including humans, and plays a central role in aging and cancer. The telomerase core enzyme is composed of the reverse transcriptase protein subunit, TERT, and a noncoding RNA, and some additional holoenzyme subunits are also essential *in vivo*. The 1157-nt *S. cerevisiae* telomerase RNA, TLC1, in addition to providing the template for DNA synthesis, is also known to serve as a flexible scaffold for holoenzyme subunits, such as the essential Est1 protein. The Est1-, Ku-, and Sm_7-binding sites in TLC1 can each be repositioned with retention of function *in vivo*. The Est1-binding region is an expansive conserved domain (108 vs. 25 and 13 nts, respectively), suggesting to us it does more than simply bind the Est1 protein. To test this, we functionally distinguished Est1 binding from potential other functions by tethering it to TLC1 via a heterologous RNA-protein binding module. Heterologous tethering rescued telomerase RNA alleles missing selected nucleotides required for Est1 binding, but not those lacking the entire 108-nt conserved region. Notably, however, telomerase function was restored to these alleles upon expression of the Est1 arm of TLC1 *in trans*. Mutational analysis shows that the Second Essential Est1-arm Domain (SEED) maps to an internal loop of the arm, which SHAPE chemical mapping and 3D modeling suggest is regulated by conformational change. Targeting of telomerase to telomeres using a Cdc13-TERT fusion protein, to bypass

Est1 recruitment, rescues Est1-binding defective TLC1 RNAs, but not SEED mutants. This provides evidence that the SEED is required to induce the extendable state of telomeres and/or contributes directly to telomerase holoenzyme mechanism. In other experiments focused on the central catalytic hub of TLC1 RNA, we have identified another essential functional feature. Telomerase RNAs contains four known core structural features: the template, template-boundary element, pseudoknot, and core-enclosing helix. We find that moving the ends of the RNA to between the template and pseudoknot is tolerated *in vitro* and *in vivo*, as is also the case within the pseudoknot or distal to the template-boundary of core-enclosing helices. However, in three of the four element-linking junctions in the central hub, circular permutations abolish telomerase activity, thus defining an Area of Required Connectivity (ARC) required to physically link the conserved elements for telomerase activity. These results provide a basic map of functional connections in the core of the yeast telomerase RNP and a framework to understand conserved element coordination in telomerase mechanism.

265A. Identification of cellular genes modulating Ty1 copy number control in *Saccharomyces cerevisiae*. ***Hyo Won Ahn***, *David Garfinkel*. Biochemistry & Molecular Biology, University of Georgia, Athens, GA.
Retrotransposons are mobile genetic elements that replicate via an RNA intermediate and exhibit many similarities to retroviruses. Ty1 is the most abundant retrotransposon in *S. cerevisiae*. Ty1 elements contain *GAG*, which encodes the capsid and *POL*, which encodes protease (PR), integrase (IN), and reverse transcriptase (RT). Since transposon movement can impact the host genome by insertional mutagenesis or chromosomal rearrangements, organisms have evolved different ways to control the level of transposition. For Ty1, our group has demonstrated that a copy number control (CNC) mechanism exists that may impair assembly and/or maturation of virus-like particles. Ty1 CNC acts post-transcriptionally and results in the loss of mature IN with a moderate decrease in RT. Recently, a N-terminally truncated form of Gag (p22) was found to play a role in CNC. Identifying host factors that modulate CNC is necessary to understand this form of transposition control. Therefore, we searched previously reported genetic screens for host cofactor genes that are required for Ty1 transposition and play a role in RNA metabolism, which is one of the highly represented gene functional groups from the cofactor collection. Ty1 transposition and protein levels were initially determined in about 50 Ty1 cofactor mutants in the *S. cerevisiae* BY4742 background. Interestingly, a *loc1* mutation enhanced the transposition defect in a CNC^+ strain when compared to WT, while there was little or no change in a CNC^- strain. Also, *loc1* mutants lacked mature integrase in CNC^+ but not in CNC^- backgrounds. *LOC1* is involved in the asymmetric localization of *ASH1* mRNA and ribosome biogenesis. Therefore, strains lacking the *ASH1* mRNA localization pathway genes (*SHE2*, *SHE3*, *MYO4*, or *PUF6*) and ribosome subunit genes (*RPL7A* or *RPS0B*) were analyzed for enhancement of CNC. Surprisingly, *rpl7a* and *rps0b* mutants showed Ty1 transposition defects similar to *loc1*, but mutants defective in the *ASH1* mRNA localization pathway genes did not. Together, these results suggest that ribosome biogenesis is critical for CNC. Future studies will address how *LOC1* affects expression, stability and function of p22, and investigate cellular factors that interact with p22.

266B. A role for the budding yeast separase, Esp1, in Ty1 element transposition. *Krystina Ho[1,2], Lina Ma[1], Stephanie Cheung[1,2], Nancy Fang[2,3], Barry Young[4], Christopher Loewen[4], Thibault Mayor[2,3],* ***Vivien Measday***[1,2]. 1) Wine Research Centre, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; 3) Centre for High-Throughput Biology, University of British Columbia, Vancouver, British Columbia, Canada; 4) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada.
Separase/Esp1 is a protease required at the onset of anaphase to cleave cohesin and thereby enable sister chromatid separation. Esp1 also has a nonproteolytic function after cohesin cleavage that promotes mitotic exit. To uncover other potential roles for separase, we performed two complementary genome-wide genetic interaction screens with a strain carrying the budding yeast esp1-1 separase mutation. We identified 161 genes that when mutated aggravate esp1-1 growth and 44 genes that upon increased dosage are detrimental to esp1-1 viability. In addition to the expected cell cycle and sister chromatid segregation genes that were identified, 24% of the genes identified in the esp1-1 genetic screens have a role in Ty element retrotransposition. Retrotransposons, like retroviruses, replicate through reverse transcription of an mRNA intermediate and the resultant cDNA product is integrated into the genome by a conserved transposon or retrovirus encoded integrase protein. We purified Esp1 from yeast and identified an interaction between Esp1 and Ty1 integrase using mass spectrometry that was subsequently confirmed by co-immunoprecipitation analysis. Ty1 transposon mobility and insertion upstream of the SUF16 tRNA gene are both reduced in an esp1-1 strain but increased in cohesin mutant strains. Securin/Pds1, which is required for efficient localization of Esp1 to the nucleus, is also required for efficient Ty1 transposition. We propose that Esp1 serves two roles to mediate Ty1 transposition - one to remove cohesin and the second based on a physical interaction with Ty1-integrase.

267C. Functional analysis of Ty1 Gag: trans-dominance and nucleic acid chaperone activity. ***Yuri Nishida***[1], *Katarzyna Pachulska-Wieczorek[2], Jessica Mitchell[1], Katarzyna J. Purzycka[2], David Garfinkel[1]*. 1) Biochemistry & Molecular Biology, University of Georgia, Athens, GA; 2) Polish Academy of Sciences, Institute of Bioorganic Chemistry, Poznan, Poland.

We are studying how Ty1 retrotransposition efficiency decreases as the number of Ty1 copies increase in the genome, a process termed copy-number control (CNC). Recent evidence indicates that a truncated form of the Ty1 capsid (p22 and a processed form called p18) located in the C-terminal half of *GAG* (CTD) is necessary and sufficient for CNC. In addition, mutations conferring resistance to p22/18 cluster just upstream of p22. Therefore, dissecting the C-terminal region of Gag should reveal more information about p22/18 trans-dominance and the nucleic acid chaperone activity displayed by this region. Expression of various portions of the CTD from the *GAL1* promoter decreased pGTy1*his3-AI* mobility in trans by 4-7 fold. However, expression of p18 from the first available AUG (AUG#1) decreased Ty1 mobility by 30,000 fold, whereas a slightly smaller version of p18 expressed from an AUG 10 residues downstream (AUG#2) decreased mobility about 100 fold. Transactions mediated by the RNA chaperone function of retroelement Gag proteins or their derivatives are essential for RNA packaging and reverse transcription. These RNA chaperones bind nucleic acids with broad specificity and facilitate their folding by destabilizing mis-folded, kinetically trapped structures and enabling the formation of the thermodynamically favoured form. *In vitro* studies have shown nucleic acid aggregation, destabilization, and rapid binding kinetics are key characteristics of RNA chaperones. Although p18 and CTD proteins both effectively aggregated nucleic acids, p18 showed significantly reduced DNA/RNA annealing activity than the CTD. We also tested protein binding to its cognate RNA. Interestingly, p18 did not bind to Ty1 RNA *in vitro*, which was in marked contrast to the CTD that bound with high affinity. Current experiments are addressing how p18 affects binding and chaperone activity of the CTD, and whether p18 directly interacts with Gag and the CTD. p22/18 may confer CNC by disrupting VLP function via binding to the CNC-resistance domain.

268A. **Regulation of sexual differentiation in *Kluyveromyces lactis* by endonuclease activity of the transposase-related gene *KAT1***. ***Naghmeh Rajaei***, *Kishore K Chiruvella, Feng Lin, Stefan U Åström.* Department of Molecular Biosciences, the Wenner-Gren Institute, Stockholm University, SE-10691 Stockholm, Sweden.
Transposable elements (TEs) have had immense impact on the structure, function and evolution of eukaryotic genomes. For example, in the development of the mammalian immune system, the Rag1/2 recombinase has evolved via domestication of a transposase gene. In this study, we identified *KAT1*, a novel domesticated DNA transposase of the hAT family in the *yeast Kluyveromyces lactis* with a biochemical activity very similar to that of Rag1/2. Kat1 triggers an adaptive genome rearrangement that results in a switch of mating type from *MAT***a** to *MAT*. Furthermore, Kat1 acts on sequences that presumably are ancient remnants of a long-lost transposable element. Therefore, Kat1 provides a remarkable example of the intricate relationship between transposable elements and their hosts. A combination of in vivo and in vitro approaches uncovered a mechanistic view on Kat1 function, establishing that Kat1 generates two DSBs in *MAT***a**. DNA hairpins were formed on one end of the DSBs whereas the spacer DNA between the DSBs was joined into a circle. Kat1 was negatively regulated by translational frameshifting and transcriptionally activated by nutrient limitation through the transcription factor Mts1. Taken together; Kat1 is a highly regulated transposase-derived endonuclease vital for sexual differentiation.

269B. A Trans-dominant form of Ty1 Gag mediates Copy Number Control of the Ty1 Retrotransposon in Saccharomyces. ***Agniva Saha***, *Jessica Mitchell, Yuri Nishida, Jonathan Hildreth, David Garfinkel.* Biochemistry and Molecular Biology, University of Georgia, Athens, GA.
Ty1 is the most abundant retrotransposon in Saccharomyces and resembles retroviruses in genome organization and replication. Ty1 has two open reading frames: GAG encodes the capsid of virus like particles (VLPs), and POL encodes protease (PR), integrase (IN) and reverse transcriptase (RT). Most eukaryotes have RNA interference mechanisms to keep transposons quiescent, since high levels of transposition may lead to insertional mutations and genome instability. However, S. cerevisiae and its closest relative S. paradoxus, lack the genes for RNAi. Instead they have evolved a mechanism termed copy number control (CNC) that inhibits Ty1 transposition in a copy number dependent manner. Recently, we discovered that Ty1 encodes its own trans-dominant inhibitor, a 22kDa protein derived from GAG. p22 is encoded by a 5 truncated mRNA, which is detected by Northern analyses of poly(A)+ RNA from wild type strains, as well as strains deleted for a major RNA degradation pathway (XRN1) or SAGA complex component (SPT3) necessary for full-length Ty1 transcription. 5 RACE mapping indicates that multiple truncated transcripts initiate about 800 nt downstream from the normal start site. In a trans-dominance assay, GAL1-promoted coexpression of p22 and a wild type Ty1his3-AI element in a Ty1-less background exhibited over a 30,000-fold decrease in mobility when compared to a control strain expressing only Ty1his3-AI. Interestingly, p22 cofractionates with VLPs, suggesting the possibility that p22 associates with full-length p45-Gag, the major capsid protein. Cells coexpressing p22 and Ty1his3-AI yield fewer VLPs, less Gag and RT, and completely lack mature IN - a hallmark of CNC. Our work strongly suggests that p22 mediates Ty1 CNC, perhaps by interacting with Gag during VLP assembly. Future studies will be aimed at understanding how p22 leads to the disruption of VLP function and ultimately prevents the accumulation of mature IN.

270C. A Tetraploid Intermediate Precedes Aneuploid Formation in Yeasts Exposed to Fluconazole. *Benjamin Harrison[1], Maayan Bibi[2], Rebecca Pulver[2], Melanie Wellington[3], Jordan Hashemi[4], Guillermo Sapiro[4],* ***Judith G. Berman***[1,2]. 1) Dept

Mol Micro & Biotept Mol Micro & Biotechnol, Tel Aviv University, Ramat Aviv, Israel; 2) Department of Genetics, Cell Biology & Development, Unversity of Minnesota, Minneapolis, MN; 3) Department of Pediatrics, University of Rochester Medical Center, Rochester NY; 4) Department of Electrical and Computer Engineering, Duke University, Durham, NC.
Candida albicans, , the most prevalent human fungal pathogen, is generally diploid. However, 50% of isolates that are resistant to fluconazole (FLC), the most widely used antifungal, are aneuploid and some aneuploidies can confer FLC resistance. To ask if FLC exposure causes or only selects for aneuploidy, we analyzed diploid strains during exposure to FLC using flow cytometry and epifluorescence microscopy. FLC exposure caused a consistent deviation from normal cell cycle regulation: nuclear and spindle cycles initiated prior to bud emergence, leading to trimeras, three connected cells composed of a mother, daughter, and granddaughter bud. Initially binucleate, trimeras underwent coordinated nuclear division yielding four daughter nuclei, two of which underwent mitotic collapse to form a tetraploid cell with extra spindle components. In subsequent cell cycles, the abnormal number of spindles resulted in unequal DNA segregation and viable aneuploid progeny. The process of aneuploid formation in *C. albicans* is highly reminiscent of early stages in human tumorigenesis in that aneuploidy arises through a tetraploid intermediate and subsequent unequal DNA segregation driven by multiple spindles coupled with a subsequent selective advantage conferred by at least some aneuploidies during growth under stress. Importantly, trimera formation was detected in response to other antifungals, in related *Candida* species, and in an *in vivo* model for *C. albicans* infection, suggesting that drug stress can induce aneuploidy in a range of fungal pathonges in vivo as well as *in vitro*. We are now testing strains carrying mutations in genes important for cell cycle progression to identify the molecular mechanism(s) necessary for trimera and subsequent aneuploid formation.

271A. DNA replication and kinetochore mutants exhibit increased DNA:RNA hybrid formation. ***A. Chan***[1]*, P. Hieter*[1,2]. 1) University of British Columbia, Vancouver, BC, Canada; 2) Michael Smith Laboratories, Vancouver, BC, Canada.
DNA:RNA hybrid formation is a significant cause of genomic instability in biological systems ranging from bacteria to mammals. To determine the scope of cellular pathways that prevent DNA:RNA hybrids in Saccharomyces cerevisiae, we performed a cytological screen of 300 mutants with chromosome instability phenotypes for elevated levels of DNA:RNA hybrids. DNA replication and the kinetochore were found to be enriched for mutants exhibiting increased DNA:RNA hybrid formation. Several DNA replication mutants were shown to result in synthetic lethality or sickness in combination with RNase H deficiency. Genome-wide profiling of DNA:RNA hybrids in *cse4-1* and *pol32* highlights various genetic features prone to hybrid formation in these kinetochore and DNA replication mutant backgrounds. These findings elucidate previously unrecognized pathways that mitigate DNA:RNA hybrid formation as well as the effect of dysfunctional DNA replication and kinetochore on the genomic DNA:RNA hybrid profile.

272B. Dosage CIN genes: A comprehensive analysis of gene dosage effects on genome stability. ***Supipi Kaluarachchi Duffy****, Sidney Ang, Phil Hieter.* Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.
Copy number aberrations (CNAs) and chromosome instability (CIN) are hallmark features of cancer, yet the link between CNAs and CIN is not well understood. Given that CIN is a predisposing event which contributes to initiation and progression of cancer, identifying CNAs that drive CIN is essential. Despite the accelerated rate of discovery of amplified and/or over-expressed genes, in comparison to loss-of-function mutations, the biological relevance of most increased copy number aberrations remain unclear. This is mainly because amplified regions often include several candidate genes.
To explore the effects of increased gene dosage on chromosome instability we performed two genome-wide screens. Our Chromosome Transition Fidelity (CTF) and a-like Faker (ALF) screens identified 262 dosage CIN (dCIN) genes, which includes 9 previously known dCIN genes such as *CLB5*, *MPH1*, *SCM3* and *CSE4*. To assess cross-species relevance we compiled a list of 168 human orthologs of yeast dCIN genes and found that 72 are amplified and/or over-expressed in cancer. One novel dCIN gene Tyrosyl-DNA phosphodiesterase 1 (Tdp1), a protein involved in DNA damage repair, is over-expressed in cancer and is a potential anticancer therapeutic target. In addition, a point mutation in Tdp1 results in spinocerebellar ataxia with axonal neuropathy (SCAN1). We show that the catalytic activity of Tdp1 is not required for dCIN, and that over-expressing the *tdp1-scan1* mutant in yeast increases chromosome instability. Synthetic dosage lethality screens over-expressing *TDP1* have identified a number of interactors, which may represent potential drug targets in tumours over-expressing hTdp1. We are attempting to recapitulate the dosage CIN phenotype of Tdp1 in cell culture. These genome-wide screens have generated a candidate list of dCIN genes that uncovers conserved pathways that control genome stability.

273C. Evidence that the extra dose of *ACT1* causes slow growth in an aneuploid strain disomic for chromosome VI through a dosage imbalance with *COF1*. ***Alex Murphy****, Keegan Gies, Kun Yang, Emma Kershisnik, Kirk Anders.* Biology Department, Gonzaga University, Spokane, WA.
Aneuploidy leads to altered phenotypes. When yeast cells gain an extra chromosome, the phenotype of the aneuploid is influenced by the effects of two phenomena: (i) a general, physiological response to excess protein expression, leading to a slowing of cell proliferation, and (ii) protein stoichiometry imbalances specific to genes on the extra chromosome (Torres *et al*., 2010). To discover specific gene dosage imbalances that contribute to chromosome-specific aneuploid phenotypes,

we have developed a model aneuploid system that carries an additional, small chromosome VI. N+1 cells disomic for chromosome VI exhibit several severe phenotypes. The most severe phenotype is inviability due to the extra dose of chromosome VI-linked *TUB2*. When a copy of *TUB2* is eliminated from the extra chromosome VI, the inviability is eliminated and an underlying slow-growth phenotype is revealed. This phenotype is quite severe in newly formed N+1 spore clones from the dissection of a 2N+1 parent, but it is unstable, giving rise to faster-growing derivatives. To discover the basis of the slow-growth phenotype, we deleted additional candidate genes from the extra copy of chromosome VI. Deletion of *CDC14* from the extra chromosome did not alter the N+1 slow-growth phenotype. Deletion of the extra copy of *ACT1*, however, did eliminate most, if not all, of the growth-rate defect. Duplication of *ACT1* is therefore necessary for the slow-growth phenotype in the N+1 cells. When we supplied normal haploids with a CEN-plasmid carrying *ACT1*, we saw a similar slow-growth phenotype in newly dissected haploids. To determine the pathway(s) by which an extra dose of *ACT1* interferes with normal growth in the aneuploid cells, we tested whether extra doses of other, candidate genes that encode actin-binding proteins can alter the aneuploid phenotype. When *COF1*, which codes for cofilin, was supplied on a CEN plasmid, the slow-growth phenotype of the N+1 was eliminated, suggesting that extra actin expression in the aneuploid may lead to slow growth through a dosage imbalance between *ACT1* and *COF1*.

274A. Evolution of chromatin accessibility and gene expression levels during the heat shock response in Saccharomyces sensu stricto yeast. ***Caitlin Connelly***, *Joshua Akey.* Genome Sciences, University of Washington, Seattle, WA.
Recent advances in functional genomics technology have revealed pervasive differences in patterns of chromatin accessibility between individuals within and between species. However, the evolution and downstream effects of heritable variation in chromatin accessibility are still not well understood, particularly under conditions other than rapid growth. Heat shock is a well-characterized stress condition in the budding yeast Saccharomyces cerevisiae that results in widespread cellular effects, including differential expression for a suite of approximately 850 genes. To better understand the evolution of the heat shock response, we used ATAC-seq (Assay for Transposase-Accessible Chromatin) to map chromatin accessibility across the Saccharomyces sensu stricto clade and RNA-seq to measure expression levels. Specifically, we assayed patterns of chromatin accessibility and gene expression levels in four strains of S. cerevisiae, and one strain each of S. paradoxus, S. mikatae, S. kudriavzevii, and S. bayanus before and after a 15 minute heat shock at 37 degrees, as well as 30 minutes after shifting the temperature back to 30 degrees. These data provide a detailed molecular portrait of the evolutionary trajectory of chromatin structure and gene expression levels in response to heat shock, facilitate the identification of polymorphisms associated with variability of chromatin accessibility and transcriptional divergence within and between yeast species, and allow us to assess the strength of purifying and positive selection acting on such variants.

275B. A High-Resolution View Of Chromatin Architecture And Transcriptional Repression At Native Telomeres in Saccharomyces cerevisiae. ***Aisha Ellahi***, *Deborah Thurtle, Jasper Rine.* Dept of Molecular and Cell Biology, Univ California, Berkeley, Berkeley, CA.
Telomeres are characterized by a specialized chromatin structure designed to prevent degradation and suppress recombination at the repetitive ends of linear chromosomes. In many organisms, this chromatin structure is repressive and associated with the transcriptional silencing of adjacent genes. In *Saccharomyces cerevisiae*, this effect, known as telomere position effect, was first described for a *URA3* reporter gene placed adjacent to an artificially truncated telomere on the left arm of chromosome VII and the right arm of chromosome V. Furthermore, silencing state of telomeres was heritable and dependent on the *S*ilent *I*nformation *R*egulator proteins: Sir2, Sir3, and Sir4. Other studies of silencing using the *URA3* reporter at native telomeres showed, however, that not all telomeres are associated with transcriptional repression and that silencing is discontinuous across the length of telomeres. Here, we present the first high-resolution look at chromatin architecture and expression state at natural telomeres free of reporter genes by utilizing ChIP-seq of Sir proteins and RNA-seq in *sir* mutants. Additionally, ChIP-seq of acetylated H4K16, a histone mark anti-correlated with silencing, was also performed to correlate heterochromatic structure with expression data from RNA-seq. Corroborating past work, we find that silencing is in fact discontinuous, and varies widely from telomere to telomere. Additionally, some telomeres are associated with sharp transcriptional boundaries, while others are characterized by gradual boundaries in transcription, perhaps due to the types of promoters present at those loci. We also found that increases in expression at some telomeres in *sir* mutants extended to several kilobase pairs beyond the boundary of Sir protein binding. This study provides the definitive analysis as to the landscape and degree of silencing at telomeres in *S. cerevisiae*, and highlights the functional variation among telomeres, befitting the accelerated sequence changes seen in these cauldrons of genetic innovation.

276C. Investigations on the nature of a nucleosomal region required for proper interactions between the transcription elongation factor Spt16 and transcribed genes in *S. cerevisiae*. *Paige Johnson, Jessalyn Tackett, Eugene Nyamugenda, Ryan Banning, Kelsi McClure,* ***Martha Kellems****, Harrison Lindley, Sarah Marshall, Andrea Duina.* Biology Department, Hendrix College, Conway, AR.

During the process of transcription elongation, RNA polymerase (Pol II) requires the assistance of a number of factors to ensure accurate and efficient production of RNA molecules. One of these factors, the highly conserved FACT complex, is thought to travel in conjunction with Pol II and to assist in the transcription process first by facilitating the removal of histone proteins in front of Pol II and then by reassembling histones into nucleosomes in the wake of Pol II passage. In previous work, we identified three histone residues - H3-L61, H4-R36 and H4-K31 - that are important for ensuring proper interactions between FACT and transcribed genes. More specifically, we found that the H3-L61W, H4-R36A and H4-K31E mutations cause a dramatic shift in FACT occupancy (as measured by ChIP experiments directed against the FACT subunit Spt16) towards the 3 ends of genes. Interestingly, these three histone residues are located in close proximity to each other on the side of the nucleosome. Based on these and other results, we hypothesize that a nucleosomal region encompassing these residues is involved in interactions with Spt16, and that these interactions are important for proper departure of Spt16 from the ends of genes following transcription. Here, we describe ongoing experiments designed to better characterize this region as it relates to Spt16 function. In one set of experiments, we are analyzing the effects of mutations of the H3-L61 residue to each of the other 19 amino acids on Spt16 distribution across the constitutively transcribed genes *PMA1* and *FBA1*. We found that most H3-L61 mutations cause a 3 shift in Spt16 occupancy at these genes, with the H3-L61W, H3-L61K, H3-L61T, H3-L61E, H3-L61H, and H3-L61Y mutations causing the most dramatic defects. In a second set of experiments, we are analyzing the effects of mutations at other residues in the nucleosomal region under investigation and to date we have identified the H3-E50A, H3-R53A, H3-E59A and H3-R63A mutations as causing marked shifts in Spt16 distribution towards the 3 regions of *PMA1* and *FBA1*. Our findings point to the precise architecture of the interface between histones H3 and H4 at the H3-L61/H4-R36 location as a critical feature of the nucleosome required for proper Spt16-chromatin interactions during transcription. In addition, our results implicate other residues located on the side of the nucleosome near H3-L61 and H4-R36 as contributors to Spt16-gene interactions.

277A. Studies addressing a possible role for TORC1 in controlling chromatin structure and transcription elongation in *S. cerevisiae.*. *Jasmine Haller[1], Mary Allison[1], Meet Modi[1], James Dornhoffer[1], Sarah Marshall[1], **Taylor McElroy**[1], Marine Boucherle[2], Anne Rufiange[2], Malena Outhay[1], Jennifer Harper[1], Amine Nourani[2], Andrea Duina[1].* 1) Biology Department, Hendrix College, Conway, AR; 2) Centre de Recherche en Cancérologie de l'Université Laval, L'Hotel-Dieu de Québec, Québec, Canada.
During transcription elongation, histones are removed in front of RNA polymerase II (Pol II) to allow Pol II access to the underlying DNA template and are then reassembled back into nucleosomes following Pol II passage. In previous work aimed at better understanding the role of histones in transcription, we isolated a histone H3 mutant - H3-L61W - that was found to confer a number of mutant phenotypes, including some indicative of chromatin and transcription elongation defects. In subsequent experiments, we discovered that conditions that impair TORC1 - a highly conserved complex involved in coupling nutrient availability with cell growth - suppress a subset of the defects seen in H3-L61W cells. These suppression effects are most evident in the context of deletion of the gene encoding the Tco89 subunit of TORC1. These and other genetic experiments presented in this poster provide compelling - albeit indirect - evidence implicating TORC1 in the regulation of chromatin structure and transcription elongation. As a way to more conclusively test whether TORC1 is involved in these processes, we have carried out chromatin immunoprecipitation (ChIP) and gene length-dependent accumulation of mRNA (GLAM) assays to directly assess the effects of deletion of *TCO89* on chromatin environments over transcribed genes and on transcription elongation. Results from ChIP assays have shown no significant alterations on nucleosome and Pol II occupancy nor on levels of specific histone modifications at several genomic locations in *tco89* cells. GLAM assays comparing wild-type and *tco89* cells did not provide evidence in support of a role for Tco89 in controlling transcription elongation. We describe ongoing studies designed to explore other chromatin processes that might be targeted for regulation by TORC1, including experiments to test if baseline histone exchange on chromatin is affected in tco89 cells.

278B. Loss of transcriptional silencing is not an obligate precursor of yeast senescence. ***Gavin S. Schlissel**, Jasper Rine.* UC Berkeley, Berkeley, CA.
Budding yeast undergo a finite number of mitotic cell divisions before they eventually senesce, and yeast replicative age is associated with myriad changes to the basic biology of the yeast cell. Specifically, aged yeast cells are reported to accumulate extrachromosomal rDNA circles, to lose silencing of auxiliary mating type genes at HML and HMR loci and in subtelomeric regions, and to mate at lower efficiency than young cells. These observations are consistent with the model that aged cells lose the function of SIR proteins, which ordinarily maintain the stability of the rDNA locus and orchestrate transcriptional silencing of HML and HMR and near telomeres. Here we revisit the question of whether SIR-protein-mediated transcriptional silencing is associated with cell age by applying a recently-developed assay that captures transient loss of silencing events as heritable changes in expression of a fluorescent reporter. We report that loss of transcriptional silencing of HML and HMR is not an obligate prelude to yeast cell senescence, suggesting that atrophy of SIR proteins is not a driving cause of cell aging.

279C. Keeping Quiet: Does heterochromatin stay silent during homologous recombination? ***Katie Sieverman***[1], *Jasper Rine*[1,2]. 1) Department of Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA; 2) California Institute for Quantitative Biosciences, University of California - Berkeley, Berkeley CA.
Heterochromatin is a dense, regional state of chromatin employed by eukaryotic cells to stably silence gene expression. Silent chromatin physically occludes transcription machinery and nucleases from DNA, yet it does not prevent the compact DNA from serving as a template for double-strand break repair. Here we explore the relationship between homologous recombination and the heterochromatic state. Mating-type switching in the budding yeast Saccharomyces cerevisiae provides a model to study the dynamics of chromatin-based silencing. Haploid yeast cells switch their mating type by initiating a programmed DSB at the *MAT* locus. Repair of *MAT* occurs via homologous recombination at either the *HML* or *HMR* locus, both of which are kept in a transcriptionally silent state. We placed the *cre* recombinase gene at the *HML* locus to monitor its heterochromatin stability. Transient lapses in silencing at *HML* allow Cre to excise an RFP cassette and activate GFP expression. Into this genetic background we introduced a non-functional copy of the *cre* sequence that contains a double-strand break. To fix the break, homology-directed repair ensues at *HML*. We can then interpret Cre expression (a cellular switch from red to green fluorescence) as a readout of *HMLs* transcriptional activity during the repair process. Using this innovative assay, we observe that approximately 30% of cells that template recombination at the silent *HML* locus lose transcriptional silencing in the process. Interestingly, many cells appear to maintain their silent state despite single-strand invasion and DNA synthesis through the locus. Our goal is to understand the mechanistic distinction behind these two classes of outcome and to identify just how the repair process disrupts silencing.

280A. Promoting a balanced acetylation state bypasses the requirement for two essential NuA4 subunits in *S. cerevisiae*. *Ana Lilia Torres-Machorro*[1,2], ***Naomi E. Frank Searle***[1,2,3], *Lorraine Pillus*[1,2]. 1) Section of Molecular Biology, Division of Biological Sciences University of California, San Diego, La Jolla, California 92093; 2) UC San Diego Moores Cancer Center, La Jolla, California 92093; 3) Biomedical Science Graduate Program, University of California, San Diego, La Jolla, California 92093.
The eukaryotic genome is packaged into chromatin, which is composed of a nucleosome unit containing DNA tightly wrapped around a histone octamer. Chromatin components are subject to various modes of post-translational regulation and have many established roles, including functions in recombination, DNA damage repair, and gene expression. Acetylation is a key post-translational modification that regulates chromatin function by modifying lysine residues on the N-terminal histone tails. Lysine acetyltransferases (HATs/KATs) and deacetylases (HDACs/KDACs) regulate acetylation through opposing enzymatic activities. In humans, Tip60 is one such KAT. Tip60 is aberrantly expressed in many human carcinomas, including down regulation in colon carcinoma and upregulation in advanced prostate cancer. In *Saccharomyces cerevisiae*, Esa1 is the homolog of Tip60 and acts analogously as the essential catalytic subunit of the NuA4 KAT complex. Esa1, like Tip60, acetylates histones H4 and H2A along with other non-histone substrates. In addition to Esa1, there are five other essential subunits in the NuA4 complex: Swc4, Act1, Tra1, Arp4, and Epl1.
Here, we report that the requirement for the essential NuA4 subunits, Esa1 and Epl1, can each be bypassed by concurrent loss of Sds3 or Dep1, non-catalytic subunits of the Rpd3L KDAC complex that function in maintaining Rpd3L complex integrity. The Rpd3 KDAC exists in three unique complexes where it is known to deacetylate many of the same targets that NuA4 acetylates, however only loss of Rpd3L can bypass *esa1* and *epl1*. We propose that the loss of the Rpd3L complex in *esa1* cells allows for viability by establishing a relatively balanced state of acetylation. We illustrate this mechanism of viability by modifying acetylation levels in the cells through modulation of additional HDACs, including Hda1, Sir2, and Hst1, along with the histone tails. While each of these additional modifications restores unique functions required for growth, such as progression through the cell cycle and response to DNA damage and stress conditions, a more balanced acetylation state of the cell underlies the viability of each bypass strain.

281B. Ubiquitin-mediated regulation of Snf1/AMPK by Ubp8 and Ubp10 in budding yeast. ***Hsiang-En Hsu***[1], *Tzu-Ning Liu*[2], *Yi-Chen Lo*[2], *Cheng-Fu Kao*[1]. 1) Institute of Cellular and Organismic Biology, Taipei, Taiwan; 2) Institute of Food Science and Technology, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan.
Ubiquitin is a small regulatory protein which is ubiquitously expressed in eukaryotes. It is inv olved in many biological processes, including protein degradation, trafficking, and gene expression. Our research is focused on two highly-conserved ubiquitin proteases, Ubp8 and Ubp10, both of which are required for deubiquitylation of histone H2B. A search for genes reported to interact with UBP8 and UBP10 revealed the yeast homologue of AMPK, SNF1. Snf1 is required when cellular glucose is limited, as it activates stress-responsive transcription factors. We used a mutant which lacks Ubp8 and Ubp10 to investigate whether Snf1 function is regulated by its ubiquitylation and degradation. The level of SNF1 mRNA in the mutant was slightly decreased compared to the wild type, whereas the level of Snf1 was dramatically decreased. The reduced level of Snf1 in the mutant had mild effects on Snf1-mediated functions. Interestingly, the remaining Snf1 in the mutant was hyper-phosphorylated during chronological aging, suggesting enhancement of Snf1 activity. We hypothesize that Snf1 a ctivity may be enhanced to compensate for defects in cells lacking both Ubp8 and

Ubp10. Surprisingly, the stability of Snf1 in the ubp8 ubp10 mutant was restored to the wild type level when either threonine 210 of Snf1 was replaced with aspartic acid (thus mimicking constitutive phosphorylation) or REG1 was deleted (thereby reducing dephosphorylation of Snf1). Our results suggest that Snf1 is protected from degradation by both Ubp8 and Ubp10. The protein level and phosphorylation status of Snf1 is tightly monitored in cells; thus, the reduced abundance of Snf1 in the ubp8 ubp10 mutant stimulates hyper-phosphorylation of Snf1, thereby protecting cells against stress and aging. Taken together, our results reveal the interaction between ubiquitylation and phosphorylation of Snf1, which fine-tunes the stability and activity of Snf1 in the face of extracellular stress.

282C. Accumulation of onco-metabolite 2-hydroxyglutarate impacts heterochromatin stability. ***Ryan Janke***, *Jasper Rine.* Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California, Berkeley, CA 94720.
Isocitrate dehydrogenase (IDH) is a highly conserved metabolic enzyme that catalyzes the conversion of isocitrate to -ketoglutarate. Mutations in two IDH isoforms, IDH1 and IDH2, occur at a high frequency in several cancers including gliomas, sarcomas, and acute myeloid leukemia. Most prevalent are missense mutations that occur at a conserved arginine residue found in the catalytic pocket of either IDH1 (R132) or IDH2 (R172) that lead to a change in the enzymatic reaction catalyzed by IDH. Rather than producing -ketoglutarate as an end product, these IDH mutants catalyze the conversion of -ketoglutarate into 2-hydroxyglutarate (2-HG). Two prominent effects have been documented in tumors that accumulate high levels of 2-HG: poor cellular differentiation and histone hyper-methylation due to 2-HG inhibition of JmjC family histone demethylases. Additionally, conditional expression of IDH mutants has been shown to have widespread effects on global gene expression profiles. However, the particular nature of how 2-HG-dependent histone hyper-methylation specifically influences transcription remains poorly studied. We hypothesize that in addition to localized effects on transcription of individual target genes, histone hypermethylation due to 2-HG accumulation may also impact larger chromatin structural domains. To study the effects of 2-HG accumulation on chromatin at a mechanistic level, we made analogous mutations of arginine 132 in the budding yeast IDH1 homolog, IDP2. As expected, this mutation resulted in histone hyper-methylation at a specific position. We find that this mutation also leads to an increase in the stability of transcriptionally silent heterochromatin regions. These results suggest that accumulation of 2-HG may have widespread impacts on chromosomal epigenetic states.

283A. Synergistic repression of FLO11 and histone methylation by the Cyclin C/Cdk8 complex and histone demethylase Jhd2p. ***Michael J. Law***, *Kerri Ciccaglione.* Rowan University-GSBS, Stratford, NJ.
Cell fate determination is dependent upon the reliable integration of extracellular signaling cues into appropriate transcriptional outputs. When starved for nutrition, Saccharomyces cerevisiae can enter one of two differentiation pathways, meiosis or filamentous growth. Filamentous growth occurs when yeast are deprived of nitrogen, fermentable carbon, or amino acids, and is characterized by enhanced cell-cell adhesion, changes in cell polarity, increases in cell length, adherence to plastic surfaces, and invasive growth into substrates. Central to transcriptional regulation underlying this process are the dynamics of post-translational histone modifications. Histones can be modified in a number of ways including acetylation, phosphorylation, and methylation. Histone H3Lys4 methylation, regulated by the opposing activities of the Set1p methyltransferase and Jhd2p demethylase, is the most well studied of the methyl marks. H3Lys4 trimethylation correlates with RNA pol II transcriptional activation. In turn, RNA pol II interacts with two genetically and biochemically distinct multiprotein complexes that are critical for its regulation, the core mediator complex and CDK8 submodule. The CDK8 submodule, consisting of cyclin C, Cdk8, Med 12, and Med13, can positively or negatively regulate transcription in a locus specific manner. How CDK8 and histone methylation are regulated enzymatically remains elusive. Here, we show that the cyclin C/Cdk8 complex and the lysine demethylase JHD2 act together to inhibit filamentous growth. Interestingly, removal of JHD2 and CNC1 results in constitutive activation of filamentation. This phenotype requires the cell surface adhesin FLO11 and two of its known activators, FLO8 and MSS11. RT-qPCR studies show that FLO11 transcription is upregulated in jhd2cnc1 mutants that are cultured in rich media. Chromatin immunoprecipitations indicate that CNC1 can inhibit H3Lys4 methylation in a kinase dependent fashion, yet independently of JHD2. Finally, ChIP-seq experiments suggest that inhibition of H3Lys4 methylation by CNC1 is restricted to specific loci, supporting earlier transcriptomics studies. Taken together, these data support a model in which the Cnc1p/Cdk8p complex can negatively regulate transcription by preventing Set1p mediated H3Lys4 methylation in a locus-specific manner.

284B. A cre-lox recombination-based assay for the study of yeast chronological aging in nutrient-rich media conditions. ***David McCleary***, *Jasper Rine.* UC Berkeley, Berkeley, CA.
Saccharomyces cerevisiae has proven to be a fantastic model organism for the discovery of genes that influence aging and age-related disease, due to its ease of study and the conservation of nutrient sensing and signaling pathways that underlie many aging processes. Despite the intense interest in yeast aging, studies have generally been performed under simple, nutrient-limited media conditions, due to experimental constraints. In studies of chronological aging, in which stationary-

phase cultures are assayed for viability over time, the failure of all chronologically aged cells in a culture to remain in a state of quiescence in complex and nutrient-rich media conditions has complicated the interpretation of aging studies on these media. We investigated the role of Sir2, a protein deacetylase implicated in the regulation of aging, in yeast chronological lifespan across a variety of complex, nutrient-rich media conditions that better reflect a niche of Saccharomyces than does the low glucose, synthetic lab culture media usually used for experiments. Our data reveal significant Sir2-dependent and Sir2-independent pathways regulating chronological lifespan that differ significantly from those observed under standard media conditions. To further investigate the mechanisms of chronological aging in these complex media conditions and determine the aging characteristics of only the subpopulation of cells that remain in a state of quiescence, we developed a cre-lox recombination-based assay for marking non-quiescent cells and their descendants to differentiate them from the chronologically aging, quiescent cells. This system exploits a *cre*-estradiol binding domain fusion construct whose expression is regulated by a daughter cell-specific promoter, developed and generously provided by the Gottschling lab. This cre fusion protein is produced only in newly formed daughter cells, and is transported into the nucleus only in the presence of estradiol. Elsewhere in the genome, loxp sites flank a *RFP* gene, which is followed by a *GFP* gene. When estradiol is added to stationary phase cultures, any new buds given off by non-quiescent cells will produce GFP protein instead of RFP following cre-mediated recombination. Quantification of RFP-expressing colonies allows for chronological lifespan measurement of only the quiescent subpopulation of cells, and thus a true measure of chronological longevity. Preliminary results demonstrate that this assay is capable of differentiating between quiescent and non-quiescent subpopulations, allowing for longevity quantification of both populations. This assay also opens up the study of chronological lifespan to high-throughput flow-cytometry analysis.

285C. *De novo* amino acid biosynthesis influences starvation-induced changes in tRNA distribution. ***Rebecca L. Hurto***[1,2], *Anita K. Hopper*[1,2]. 1) Molecular Genentics, Ohio State Univ, Columbus, OH; 2) The Center for RNA Biology, OSU.
Transfer RNAs that are transcribed in the nucleus undergo numerous modifications in the nucleus and cytoplasm before functioning in cytoplasmic translation. While tRNA transport was initially thought to be unidirectional (nucleus to cytoplasm), several studies have demonstrated that tRNA moves bidirectionally between the nucleus and cytoplasm in *S. cerevisiae* and mammalian cells. The intracellular distributions of tRNA change in response to acute loss of various nutrients. The loss or absence of amino acids (aa) is one type of stress that may occur in nature. Cells posses several pathways that respond to the loss of available aa. One of the responses is to increase expression of the amino acid biosynthetic pathway proteins via the general amino acid control pathway (GCN). We previously observed tRNA nuclear accumulation in response to acute loss of all 20 aa. The yeast strains utilized for these studies harbored mutations in one or more aa biosynthetic pathways, causing them to be auxotrophic for some aa and prototrophic for others. Here, we determined how the distribution of tRNA is affected when cells only lose one or more aa that they can replace via *de novo* synthesis. Loss of a single aa that cells are able to synthesize did not result in a detectable change in tRNA nucleus-cytoplasm distribution. In contrast, acute loss of 17 aa that cells are able to synthesize resulted in a transient nuclear accumulation for most of the tRNA isoacceptors tested. The accumulation is transient because the intercellular distribution of tRNA returns to a distribution similar to the cells grown in nutrient replete conditions after 120 min. Nuclear accumulation of tRNA was observed after 120 and 180 min of acute loss of the same 17aa in *gcn4* cells, which lack the aa starvation-induced transcription factor. Thus, the return of tRNA to pre-starvation distributions depends on de novo synthesis of the amino acids. Cumulatively, the data indicate that acute loss of aa from the media induces nuclear accumulation of tRNA until the missing amino acids are replaced by *de novo* synthesis.

286A. The prion-like domain of the RNA-binding protein Ssd1 regulates the nuclear barcoding of Ssd1 to define its cytoplasmic destiny. ***Cornelia Kurischko***, *James R. Broach.* Biochemistry and Molecular Biology, College of Medicine, Penn State University, Hershey, PA.
While nuclear RNA imprinting is well studied, the nuclear barcoding or marking of RNA-binding proteins that defines their cytoplasmic fate is an emerging field of studies. Ssd1 is a good model to study the multiple roles of RNA-binding proteins in the life of their target mRNA, from transcription and maturation to translation and decay. Ssd1 contains two putative prion-like domains in its N-terminus. Ssd1 prion-like domain I specifically binds the Ser2/Ser5 phosphorylated RNA polymerase II large subunit C-terminal domain. This is consistent with the co-transcriptional binding of Ssd1 to its target RNAs. Additionally, Ssd1 is involved in the polarized localization of SRL1 mRNA. Ssd1 also interacts with components involved in all steps of mRNA degradation by binding Not1, Dcp2, Pat1 and Xrn1 and colocalizing with components of both P-bodies (PB) and stress granules (SG) upon glucose deprivation. Here we show that the prion-like domain I is essential for PB and SG localization of Ssd1, which also depends on PB components such as Edc3. PB proteins Edc3, Pat1 and Not1 are also crucial for the nuclear export of Ssd1, indicative of a new role for these proteins. We conclude that Ssd1 is marked in the nucleus for either translational competent mRNPs or for SG- and PB-targeted mRNPs for mRNA storage and decay. This process also depends on the status of the Cbk1 phosphosites in the N-terminus of Ssd1. Non-phosphorylatable Ssd1 ($Ssd1^{S/T9A}$) constitutively exits the nucleus and localizes to PB independently of Edc3. A second NLS in the prion-like domain I is likely also regulated by phosphorylation of a Cbk1 site. A mutated constitutive

NLS (aa 417-427) prevents Ssd1 from nuclear localization and results in the formation of large Edc3-independent granules. It also causes sensitivity to various growth conditions. Both the large aggregates as well as the growth defects are suppressed by also mutating Cbk1 phosphosites to alanine, supporting the model that the second NLS is activated by dephosphorylation. In summary, Ssd1 prion-like domain I has functions in nuclear localization, cotranscriptional binding to its target RNAs, and cytoplasmic aggregate formation. Its functions are regulated by phosphorylation of the Cbk1 sites.

287B. Ribosome Subunit Biosynthesis Crosstalk During Repression of Ribosomal Protein Synthesis. ***Brian K. Gregory***, *Lasse Lindahl.* Biological Sciences, UMBC, Baltimore, MD.
The ribosome is the dynamic organelle responsible for the translation of mRNA into protein. Ribosome biogenesis is a complex, hierarchical process that originates in the nucleolus with the transcription of the pre-rRNA. Cleavage, modification and folding of the nascent transcript as well as the incremental addition of the 79 ribosomal proteins are aided by the transient interaction of over 200 non-ribosomal protein factors. The precursor particles, which will later give rise to the 40S and 60S subunits, follow separate maturation paths from the nucleolus, through the nucleoplasm and finally to the cytoplasm. Disruption of normal ribosome biogenesis results in ribosomal/nucleolar stress, which can affect cell viability and has been implicated in several human diseases (ribosomopathies). Several groups have investigated the effects of such stress on rRNA processing and the assembly of the precursor particles. These studies, however, have largely focused on the individual subunits. Our current inquiry seeks to investigate possible crosstalk between large and small subunit precursor particles during biogenesis; i.e. how does repression of 40S synthesis affect the 60S biogenesis and vice versa. In particular, rRNA intermediates, precursor stability and accumulating particles are being analyzed upon repression of specific ribosomal protein synthesis. We do indeed see changes in the processing pattern of one subunit when the synthesis of a protein from the other subunit is repressed. These changes can be seen in our Western and Northern blot analysis as well as sucrose gradient profiling. Several other approaches to understanding this nucleolar stress in yeast are currently under development, and current progress will be presented.

288C. Mitochondria outer membrane proteins are required for the proper function and localization of tRNA splicing endonucleases in Saccharomyces cerevisiae. ***Yao Wan***[1,2], *Jingyan Wu*[1,2], *Anita Hopper*[1,2]. 1) Molecular Genetics, The Ohio State University, Columbus, OH; 2) Center for RNA Biology, The Ohio State University, Columbus, OH.
tRNAs function to bring amino acids to ribosomes during protein synthesis. In yeast Saccharomyces cerevisiae, splicing of pre-tRNAs is essential for the production of 10 families of tRNAs. Intron removal is catalyzed by the heterotetrameric tRNA splicing endonuclease (SEN) complex, which is located on the cytoplasmic surface of mitochondria. Sen2 and Sen34 are the catalytic subunits of the SEN complex, whereas Sen15 and Sen54 are thought to serve a structural role. However, how and why SEN subunits assemble on the surface of mitochondria is unknown. We previously showed that when SEN subunits are relocalized in the nucleus, tRNA splicing, nuclear export, and aminoacylation are normal, but cells are inviable. Thus, there is an unknown requirement for the SEN complex to reside on mitochondria surface. Our recent genome-wide screen to search for all yeast gene products involved in tRNA biology identified two mitochondrial proteins, Tom70 and Sam37. Deletion of TOM70 or SAM37 cause pre-tRNA splicing defects and the accumulation of end-matured, intron-containing tRNAs. Tom70 is a component of the translocase of the outer mitochondrial membrane (TOM) complex and Sam37 stabilizes the sorting and assembly machinery (SAM) complex which functions in inserting beta-barrel proteins into the mitochondria outer membrane. In tom70 cells, a portion of the Sen15 and Sen54 pools are relocated from the mitochondria surface to the cytoplasm, but Sen2 remains associated with mitochondria. Thus, via direct or indirect interactions, Tom70 is required for the proper localization, assembly, and function of the SEN complex on mitochondria. Our results show that appropriate assembly of the SEN complex on mitochondria is necessary for efficient pre-tRNA splicing. These data provide the first information for how the SEN complex locates to and assembles on the mitochondrial surface.

289A. Determination of in vivo RNA kinetics using RATE-seq. ***David Gresham***, *Benjamin Neymotin, Rodoniki Athanasiadou.* Center for Genomics and Systems Biology, Department of Biology, New York University, New York, NY.
The abundance of a transcript is determined by its rate of synthesis and rate of degradation; however, global methods for quantifying RNA abundance cannot distinguish variation in these two processes. We have developed a method called RNA Approach To Equilibrium sequencing (RATE-seq), which uses in vivo metabolic labeling of RNA with 4-thiouracil (4sU) and approach to equilibrium kinetics, to determine RNA degradation and synthesis rates. RATE-seq does not disturb cellular physiology, requires minimal normalization, and can be readily adapted for studies in most organisms. We demonstrate the use of RATE-seq to estimate genome-wide kinetic parameters for coding and non-coding transcripts in Saccharomyces cerevisiae. We are applying RATE-seq to study the regulatory role of mRNA degradation in remodeling of the transcriptome in response to extracellular conditions.

290B. Global analysis of 5'-hydroxyl RNA surveillance and turnover. ***Sally Peach***, *Kerri York, Jay Hesselberth.* University of Colorado School of Medicine, Department of Biochemistry and Molecular Genetics, Program in Molecular

Biology, Aurora, CO.
Many RNA decay products have 5'-hydroxyl (5'-OH) termini that are not detected by conventional cloning schemes. We developed a method to identify 5'-OH RNA fragments from cellular RNA by ligating an adaptor to 5'-OH RNA with E. coli RtcB RNA ligase, which uses a 5'-OH as a nucleophile during ligation. Ligated 5'-OH RNA fragments are reverse transcribed, PCR amplified and analyzed by massively parallel DNA sequencing. We validated the method in budding yeast by specific capture of ribosomal RNA 5'-ends only after their conversion from 5'-phosphate to 5'-OH by phosphatase treatment. 5'-OH RNA fragments derived from polyadenylated budding yeast mRNAs included multiple distributed fragments near the 5' or 3' ends of specific mRNAs (MDH1 and TDH1) and fragments likely derived from single cleavages at specific sites in mRNAs (ADE8 and YKT6). One of the specific cleavages included a 5'-OH mRNA fragment created 2 nt downstream of the ubiquitin coding region within the open reading frame encoding the ubiquitin-Rps31 fusion protein, suggesting that this 5'-OH fragment may be generated during co-translational ubiquitin processing. Phosphorylation of 5'-OH RNAs creates 5'-phosphate RNA substrates for the yeast 5'-3' exoribonucleases Rat1 and Xrn1. During the unfolded protein response, the ER-localized Ire1 endoribonuclease cleaves the Hac1 mRNA, yielding cyclic-phosphate and 5'-OH RNA fragments that are ligated by Trl1 tRNA ligase to produce mature Hac1 mRNA and activate downstream target genes. Surprisingly, Hac1 cleavage fragments have previously been identified with 5'-phosphates in vivo, suggesting that the kinase activity of Trl1 phosphorylates these RNAs, but does not always complete ligation. In support of a decoupling of phosphorylation and ligation by Trl1, we found that Xrn1 suppresses the unfolded protein response by degrading phosphorylated 5'-OH Hac1 fragments, indicating that turnover of these RNA fragments competes with their ligation to tune the activation level of the unfolded protein response.

291C. Genome-wide study of the interdependence between the cellular growth rate and mRNA turnover. *J. Garcia-Martinez[2], G. Ayala[3], D.A. Medina[1], R. Gonzalez[4], J. Warringer[5], **J. E. Perez-Ortin**[1].* 1) Departamento de Bioquimica y Biologia Molecular, Universitat de Valencia. Spain; 2) Departamento de Genetica, Universitat de Valencia. Spain; 3) Departamento de Estadistica e Investigacion Operativa, Universitat de Valencia. Spain; 4) Instituto de Ciencias de la Vid y del Vino. Logrono. Spain; 5) Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden.
Transcription is necessary for cell growth. Although most of the energy expenditure of growing yeast is devoted to protein biosynthesis [1], a transcription cost also exists as 75% of transcription in exponentially growing yeast cells is performed by manufacturers of the translation machinery, that is RNA polymerases I and III. Therefore, it seems logical that total transcription rate (TR) depends on the actual growth rate (GR) of the cell cultures. However, the dependency on GR of the RNA polymerase II TR has not been investigated. Here we use genome-wide data collected from 42 different growth conditions and mutant strains for measuring total nascent RNA pol II TR [2] to show that there is a direct relationship between RNA pol II TR and the cell GR. Global mRNA stability (RS), on the contrary, is inversely related to GR. Several functional gene groups related to protein biosynthesis show a stronger positive dependence, whereas genes related to respiration and mitochondrial functions, controlled by Puf3 RNA binding protein, show an inverse relationship with GR. Meta-analysis of data published by another group using different methods for TR and RS evaluation [3] reinforces our conclusions and shows that Ribosome biogenesis and Ribosomal protein genes use both TR and RS to increase mRNA levels whereas Puf3-regulon genes use mainly RS to adapt mRNA levels to the cell GR. REFERENCES [1] J.R. Warner (1999). Trends Biochem. Sci. 24:437-40. [2] J. García-Martínez et al. (2004). Mol. Cell 15:303-13. [3] M. Sun et al. (2013). Mol. Cell 52:52-62. ACKNOWLEDGEMENTS J.E.P-O. is supported by grants from the Spanish MCINN and the European Union funds (FEDER) (BFU2010-21975-C03-01), and from the Regional Valencian Government (PROMETEO 2011/088).

292A. Systematic Identification and Analysis of pre-mRNA Splicing Regulators in *Saccharomyces cerevisiae*. ***H. Wang**[1,2], T. Chang[1,2], M. Hwang[1,2].* 1) Academia Sinica, Taipei, Taiwan; 2) Genome and Systems Biology Degree Program, NTU, Taipei, Taiwan.
Splicing regulatory network is one of primary contributors to both proteomic complexity and control of gene expression levels in response to the changes of physiological conditions. An important long-term goal in the splicing field is to determine a set of rules that can be used to predict the splicing pattern of any primary transcript. However, interactions between splicing regulators and splicing events have traditionally been identified in slow and small scale manner. To address the problem, I propose here to develop a high-throughput screening method that uses a dual-color fluorescent reporter system to systematically quantify pre-mRNA splicing. The reporter system contains two color fluorescent proteins, GFP and RFP, which are fused to intron-containing and intron-eliminated (I) of a specific query gene respectively. The intron-eliminated (I) construct is designed as an internal control to avoid falsely identified regulators. This reporter system can combined with genome deletion collection, gene overexpression library, or small-molecule library to high-throughput screen proteins or compounds that can modulate splicing of certain transcripts under various conditions and cellular states. In the proposal, I will use this screening system to identify splicing regulators involving in inefficient splicing events and construct an interaction network based on these splicing regulators. The ability to perform

quantitative single-cell analysis of splicing in a high-throughput scale will help to understand the splicing regulatory network and identify compounds that could be used to treat pathogenic splicing abnormalities.

293B. In search of PP2A/Cdc55 targets involved in stress induced transcription. *Jessica Ferrari, Wolfgang Reiter, David Hollenstein,* ***Gustav Ammerer***. Dept Biochem & Cell Biology, University of Vienna, Vienna, Austria.
The protein phosphatase 2A/Cdc55 has been shown to modulate activation of the so-called environmental stress response which is mainly mediated by the transcription factor Msn2. Indeed, we have documented a close correlation between transcription of Msn2-responsive genes and their temporal dependence on PP2A/Cdc55 during osmotic stress [1]. This dependence can be observed with constitutively nuclear Msn2 suggesting that the role of PP2A/Cdc55 in the stress response has to be at the nuclear level. Additional data indicated that the function of PP2A is not needed at the level of initiation but for the maintenance of the transcriptional response. However, the detailed mechanisms of how PP2A/Cdc55 causes this effect are still unknown. We therefore searched for possible targets of PP2A/Cdc55 by quantitative mass-spectrometry, comparing the phosphorylation levels of the yeast proteome in wild type and cdc55 strains under different environmental conditions. Validation of putative PP2A/Cdc55 nuclear targets has been initiated via MS analysis of purified proteins and by protein-proximity assays. From the current set of likely candidate targets, we have focused our attention on monitoring the precise phosphorylation pattern of Med15/Gal11, a component of the mediator complex as well as the two histone-demethylases, Rph1 and Gis1. [1] W.Reiter et al. Mol. Cell. Bio. 2013 p.1057-1072.

294C. Regulation of stress induced gene expression in yeast. ***Vasudha Bharatula***[1], *Nils Elfving*[2], *Razvan Chereji*[3], *Stephan Bjorklund*[4], *Alexandre Morozov*[3], *James Broach*[1]. 1) Department of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA 17033, USA; 2) Department of Medical Biochemistry and Biophysics, Umeå University, Umeå 901 87, Sweden; 3) Department of Physics and Astronomy, Rutgers University, Piscataway, NJ 08854, USA; 4) BioMaPS Institute for Quantitative Biology, Rutgers University, Piscataway, NJ 08854, USA.
Yeast cells exhibit large scale transcriptional changes in response to environmental stress which enables them to adapt and survive adverse conditions. Transcriptional changes are primarily mediated by a pair of zinc finger transcription factors Msn2/4 which induce or repress a subset of their target genes. Stress induces dephosphorylation and subsequent nucleo-cytoplasmic shuttling of Msn2, which translates into changes in gene expression. We studied the impact of Msn2 oscillations on target gene expression by performing microarrays on an estradiol-inducible, Msn2-WT strain and a constitutively nuclear Msn2 phospho-mutant strain (Z_4EV Msn2-6A). We observed that target genes display differential expression kinetics i.e. fast and slow responding genes depending on the nuclear occupancy of Msn2. *ALD3* and *TKL2* were identified as slow responding genes whereas, *DDR2*, *DCS2*, *RTN2* and *HXK1* were some of the fast responding genes. Constitutive nuclear occupancy of Msn2, as observed in the Z_4EV Msn2-6A strain reduced the induction time for slow responding genes. Our results are in agreement with the Hansen and OShea study, where some genes were found to be responsive to oscillations of Msn2 and others preferred prolonged Msn2 nuclear occupancy. In our study, no correlation was observed between kinetics of gene expression and nucleosome remodeling at promoter regions of slow and fast responding genes. We are continuing our studies on Msn2 binding by ChIP-seq and nucleosome remodeling to elucidate a possible mechanism behind the response kinetics we observe. Interestingly, along with changes in gene expression we also observe the stress induced growth arrest phenotype in our estradiol-inducible Msn2 system. Several cell-cycle regulating genes were induced in our Z_4EV Msn2-6A strain. We are studying how cells regulate cell cycle entry and exit during stress and the possible role of Msn2 in this process.

295A. Natural yeast promoter variants harbour different levels of transcriptional-mediated noise. *Jian Liu*[1], *Marlène Vuillemin*[1], *Hélène Martin-Yken*[1], *Frédéric Bigey*[2], *Sylvie Dequin*[2], *Jean-Marie François*[1], ***Jean-Pascal Capp***[1]. 1) LISBP, INSA/Univ. of Toulouse, Toulouse, France; 2) INRA, UMR 1083, Montpellier, France.
Increase in phenotypic fluctuations through noise in gene expression is a relevant evolutionary strategy in selective environments. Differences of transcriptional-mediated noise between S.cerevisiae strains could have been selected for thanks to the benefit conferred by gene expression heterogeneity in the stressful conditions experimented by technological strains for instance. Here we used a genome wide approach to identify promoters conferring high level of noise in the oenological S.cerevisiae strain EC1118. Genomic fragments were cloned in a promoterless GFP-coding vector to create a GFP-fused genomic library. This library was enriched for promoters with high noise by several rounds of fluctuating selection for GFP expression using FACS. Many promoters of genes involved in stress response or related to other environmental factors have been found. We chose 8 promoters containing genetic variations compared to their counterpart in the lab strain S288c because we hypothesized that these variations possibly generate differences of noise. Each version of these promoters has been fused to GFP, and integrated in the LEU2 locus in S288c and EC1118 to measure mean and noise. In general, the industrial promoters conferred higher mean expression associated to lower noise level, or inversely. But this relationship was not observed with CUP1 and CAN1. Here the higher mean expression with the industrial promoter was not associated to lower noise, meaning that it could confer higher noise at the same mean expression level. The inducibility of CUP1 allowed measurement of noise with different induction conditions giving the same mean

expression for both versions. We observed higher noise with the industrial version in this case. Thus we identified for the first time natural yeast promoter variants harbouring different levels of transcriptional-mediated noise. Moreover the difference is only observed in the industrial background, showing the combined influences of cis and trans factors in generating noise differences. Phenotypic consequences of these differences will be discussed.

296B. Role of Chromosomal Looping in the Transcriptional Regulation of Molecular Chaperone Genes in *Saccharomyces cerevisiae*. ***Surabhi Chowdhary***, *David Gross.* Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, Shreveport, LA.
It is well established that in metazoans, chromatin looping plays a significant role in gene regulation by bringing distal regulatory elements in close proximity to each other as well as to the core promoter. However, its role in *S. cerevisiae* is less clear. Evidence that DNA looping may have roles in this organism in transcriptional memory, targeting of chromatin remodeling enzymes, suppression of anti-sense transcription, coupling transcription to mRNA export and/or enhancement of transcription termination has been reported. Yet in each of these cases, the aforementioned activity has been observed for a few select genes. Thus, it is still an open question if DNA looping in *S. cerevisiae* is a general mechanism, and the specific biological role(s) that DNA looping may have at particular genes. We are interested in understanding the mechanisms by which Molecular Chaperone (aka Heat Shock Protein [HSP]) genes are regulated. These genes, and others whose expression is vigorously induced in response to thermal, oxidative and chemical stress, are under the control of a highly conserved transcriptional activator,Hsf1, whose dysfunction in humans has been linked to neurodegenerative diseases and cancer. We wish to know if the model *HSP* genes namely,*HSP82, HSP104, SSA4 & ZPR1*, engage in gene looping (tethering promoter/UAS with 3-end/terminator regions) in response to heat shock, and if so,[i]what are the kinetics of the formation of a gene loop?;[ii]what role,if any, does the transcriptional co-activator, the Mediator, play in facilitating the formation of gene loops?;[iii]what role, if any, does gene looping play in regulating *HSP* gene transcription, nuclear localization and in blocking anti-sense transcription? We are also interested in the general issue of 3D chromatin structure associated with *HSP* genes and the possibility that they may associate into factories upon exposure to acute heat stress. Current experiments are aimed at standardizing the technique of chromosome conformation capture (3C) for confirming the presence of gene looping at *HSP* genes. We have optimized the use of restriction enzyme, Taq I, for examining looping between the promoter and the terminator regions of *HSP82 & HSP104* in chromatin isolated from both, control and heat shock-induced cells. Specifically, we found that digesting formaldehyde cross-linked chromatin for 7 h using 200 U of Taq I at 60C was required for obtaining maximal cleavage. Our next goal is to establish optimal conditions for ligation followed by locus specific multiplex PCR analysis in order to test for the presence of a 3C signal.

297C. Transciptional Profiling of Budding Yeast Biofilm Suppressors. ***Gareth Cromie***[1], *Zhihao Tan*[1,2], *Eric Jeffery*[1], *Michelle Hays*[2], *Cecilia Garmendia*[3], *Aimée Dudley*[1,2]. 1) Dudley Group, Pacific Northwest Diabetes Research Inst, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA USA; 3) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France.
Many microorganisms, including fungi, can transition between growth in planktonic and sessile, biofilm forms. Fungal biofilms display increased anti-fungal drug resistance and an ability to colonize surfaces, including human tissues and medical devices. *Saccharomyces cerevisiae* strains having the complex, wrinkled, fluffy colony phenotype display the cell-cell adhesion and extracellular matrix production that characterize microbial biofilms and provide a model for understanding these structures. We previously identified aneuploid yeast strains where gain of a single chromosome in a haploid background suppressed the fluffy phenotype. Extending this work, we have now identified suppressors of the fluffy colony phenotype in an overexpression screen using a barcoded low copy-number plasmid library containing 4981 ORFs controlled by their native promoters. This screen identified five genes, including four genes not previously implicated in yeast biofilm regulation. RNA-seq analysis of colonies overexpressing each of these genes, as well as two disomies that also suppress the fluffy phenotype, identified a large group of genes showing differential expression in the smooth strains relative to the original fluffy strain. Taken together, the differential expression pattern of genes across this dataset may be informative for understanding environmental conditions that may be created or alleviated by growth within a fungal biofilm.

298A. Unanticipated complexity at the *GAT1* locus. ***I. Georis***[1], *R. Rai*[2], *J.J. Tate*[2], *T.G. Cooper*[2], *E. Dubois*[1]. 1) Institut de Recherches Microbiologiques J.-M. Wiame, Brussels, Belgium; 2) Dept. Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA.
The environmental nitrogen supply of *S. cerevisiae* cells ranges from luxurious to nonexistent. To cope with such widely varying conditions, these cells finely regulate the nitrogen-responsive GATA family transcription factors: Gln3, Gat1, Dal80 and Gzf3. Activities of the transcription activators Gln3 and Gat1 are particularly critical in the adaptive responses. Too little Gln3 and Gat1 places the cell at risk of starvation, whereas too high levels are equally detrimental. To understand how such sophisticated regulation is achieved, we previously investigated Gat1 protein levels using varying conditions of nitrogen availability. These studies revealed the presence of four Gat1 isoforms, two of which were produced

at a low constitutive level irrespective of the nitrogen supply. The other two (derived from a C-terminal truncated Gat1-Myc[13] fusion followed by a heterologously added transcription terminator) were produced at similarly low levels in repressive conditions (glutamine as nitrogen source), but at very high levels in derepressive conditions (proline as nitrogen source). Surprisingly, both constitutive and derepressible production of the Gat1 isoforms was dependent on Gln3. Since there was no previously reported example of Gln3 supporting both constitutive and derepressible gene expression, we investigated the regulation of Gat1 production more thoroughly. We first queried whether the contrasting Gat1 levels resulted from transcriptional or post-transcriptional regulation. Using qRTPCR, we observed a sharp decrease in Gln3-dependent mRNA levels proceeding from the 5 to the 3 end of the *GAT1* gene. This suggested the existence of a premature termination event and explained the non-parallel production of *GAT1* mRNA and Gat1 protein. Also consistent with these findings, RACE PCR analyses identified three different 5 ends, correlating with the protein species observed in western blots, and two different 3 ends, confirming possible premature termination. Together, these data suggest that *GAT1* transcription is initiated in a nitrogen-responsive manner. However, in most instances the mRNA chain is prematurely terminated thereby accounting for the low, constitutive levels of Gat1 protein. We are currently investigating the function of the different Gat1 isoforms as well as the role and mechanism of premature termination in nitrogen-responsive gene expression. Support: NIH GM-35642 (TC, JT, RR); COCOF & FRFC 2.4547.11 (IG, ED).

299B. Selective interaction of RNA-binding proteins with transcript isoforms shapes the post-transcriptional life of mRNA. ***Ishaan Gupta***[1], *Bernd Klaus*[2], *Sandra Cluader-Münster*[1], *Aino Jäverlin*[1], *Raeka Aiyar*[1], *Vicente Pelechano*[1], *Lars Steinmetz*[1,3,4]. 1) 1European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany; 2) European Molecular Biology Laboratory (EMBL), Centre for Statistical Data Analysis, Meyerhofstrasse 1, 69117 Heidelberg, Germany; 3) Stanford University School of Medicine, Department of Genetics, Stanford, CA 94305, USA; 4) Stanford Genome Technology Center, 855 California Ave, Palo Alto, CA 94305, USA. Extensive variation in the length of untranslated regions (UTR) of transcripts arising from the same gene have been reported, yet the functional consequences this variation remain largely unexplored. Here we systematically discriminate between the post-transcriptional fates of overlapping coding and noncoding transcriptional events from each genic locus using a novel genome-wide, nucleotide-resolution technique to quantify the halflives of 3' and 5' transcript isoforms in yeast. Our results reveal widespread differences in stability among isoforms for hundreds of genes in a single condition, and that variation of even a few nucleotides in the UTR can affect RNA turnover. Further, we find that due to different length of UTRs two isoforms of the same gene can have a different repertoire of RNA binding proteins (RBP) interacting with a transcript through RBP motifs. We find that not just the presence but the location of a RBP motif in the UTR affects the post-transcriptional fate of mRNA such as mRNA turnover. We identified that some motifs like PUF3 are destabilizing in both the 3' and 5' UTR while other motifs stabilize/destabilize depending on whether they are present in the 3' or the 5' UTR . We confirmed the role of RNA-protein interactions in conditioning isoform-specific stability, demonstrating that RBP PUF3 binds and destabilizes specific polyadenylation isoforms. Our findings indicate that although the functional elements of a gene are encoded in the DNA sequence, the structure of the transcribed molecule determines the incorporation of these elements into RNA which shapes the post-transcriptional life of a transcribed gene.

300C. Spinning disk confocal microscopy of galactose-responsive intra-nuclear clusters of the Gal80 protein in live cells of Saccharomyces cerevisiae. ***James E. Hopper***[1,2], *Sudip Goswami*[1], *Onur Egriboz*[1], *Kathleen Dotts*[1]. 1) Dept Molecular Genetics, Ohio State Univ, Columbus, OH; 2) Dept of Chemistry and Biochemistry, Ohio State Univ, Columbus OH. Previously we reported the discovery of intra-nuclear clusters of GFP/YFP-tagged Gal80, the inhibitor of Gal4. The clusters were shown to dissipate upon galactose- triggered interaction between Gal3 and Gal80, an event known to relieve Gal80 inhibition of Gal4 and lead to Gal4-mediated activation of GAL gene transcription. Nearly 70% of cells show 2 to 3 such clusters. Photon counting yields estimates of 24 to120 Gal802YFP molecules per cluster. Cluster topology appears highly dynamic, consistent with Gal80s tendency to form oligomers. (Melcher 2001; Egriboz 2013). Here we report new results indicating that Gal80 clusters nucleate from Gal4p-binding UASgal sites and depend on Gal4. In gal4 deletion cells we observe no Gal80 clusters with wt Gal80, but can resurrect Gal80 clusters if Gal80 is fused to the Gal4 DNA binding domain. We find a statistically significant tendency for one of the 2 to 3 clusters to co-localize with the LacO array-marked native chr II GAL1-GAL10-GAL7 locus, a locus that has 6 UASgal sites. Deletion of that locus causes loss of Gal80YPF cluster co-localization with the linked 64X lacO array. Consistent with this we observe co-localization of at least one Gal80 cluster with loci to which we ectopically add 4 Gal4 UASgal sites. To probe possible functionality of clusters we constructed a strain expressing PP7CFP and Gal802YFP and containing the GST gene expressed from the 4UASgal-associated GAL1 promoter. We inserted 24 PP7 binding sites into the 3 UTR of GST, and located the construct close to the 64X lacO array markable with lacI mcherry. With this strain we could simultaneously monitor Gal802YFP cluster co-localization with the PGAL:GST gene and the transcriptional state of GST. We observed Gal802YFP cluster co-localization with the LacO array when the PGAL:GST gene was not transcribed (Gal80-inhibitied) but not when it was transcribed (+galactose). This latter correlation is consistent with the hypothesis that Gal80 clusters represent oligomeric assemblies functioning to tightly repress the GAL1-GAL10-GAL7 genes under non-galactose-inducing conditions.

301A. Controlling isoprenoid production using a microaerobic-responsive switch in yeast. ***Hanxiao Jiang***, *Robert Dahl, Adam Meadows.* Amyris Inc. , 5885 Hollis St. Suite 100, Emeryville, CA 94608.
Amyris has engineered yeast to aerobically produce high levels of isoprenoids (e.g. farnesene) by overexpressing the genes encoding the native mevalonate pathway. The burden of constitutively overexpressing pathway proteins and producing isoprenoids at high yields significantly slows cell growth rate and leads to longer fermentation processes to reach target cell densities. In order to decouple growth and farnesene production, an industrially scalable and affordable genetic switch is desired. Here we describe a genetic switch using a microaerobic-responsive promoter to drive the transcription factor GAL4 and control the expression of mevalonate pathway genes. As oxygen is the input to control pathway expression, the switch requires no additional inducer or repressor to control gene expression and the mevalonate pathway responds only once target cell density has been achieved and the fermentation becomes micro-aerobic, making it ideal for industrial processes. Yeast strains with this genetic switch not only grow faster during cell population expansion, but also are more stable and produce higher farnesene yields in production fermentations.

302B. Changes in RNA Polymerase II catalytic activity influence transcription start site utilization on a global scale. ***H. Jin***[1], *S. Schwartz*[2], *I. Vvedenskaya*[3], *I. Malik*[1], *C. Qiu*[1], *B. Nickels*[3], *C. Kaplan*[1]. 1) Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA; 2) Texas Agrilife Research, Genomics and Bioinformatics, College Station, TX, 77840, USA; 3) Waksman Institute, Rutgers University, Piscataway, New Jersey 08854, USA.
Transcription initiation by RNA Polymerase II (Pol II) is an essential step in gene expression and regulation. Promoter recognition and transcription start site (TSS) selection by Pol II require the activity of a number of conserved factors. In *Saccharomyces cerevisiae*, TSSs are further away from recognizable promoter elements than in higher eukaryotes. Almost all *S. cerevisiae* genes utilize clustered, multiple TSSs, which is similar to the majority of promoters in metazoans such as *Drosophila melanogaster* and vertebrates. In *S. cerevisiae*, mutations in initiation factors can confer altered TSS selection/utilization. Alterations of the *S. cerevisiae* Pol II trigger loop (TL), an essential region within the Pol II largest subunit Rpo21/Rpb1 that plays major active site roles, have been shown to alter both Pol II catalytic activity *in vitro* and TSS utilization at model genes *in vivo*. We performed genome wide detection of presumptive TSSs by SoLiD sequencing of capped RNA 5 ends to investigate effects of altered Pol II activity on TSS distribution. We show that TL mutants confer TSS shifts globally at all classes of yeast promoter, consistent with the types of shifts observed for previously tested model genes such as *ADH1*. We also examined alleles of Pol II General Transcription Factors (GTFs) TFIIB and TFIIF to compare the effects of GTF mutants in TSS utilization to those of TL mutants. Effects of TL and GTF mutants were similar. A *sua7-58A5* (TFIIB) strain exhibited global TSS changes highly similar to those of TL mutants that confer reduced catalytic activity, while a *tfg2146-180* (TFIIF) strain exhibited TSS changes highly similar to those of Pol II TL mutants with increased catalytic activity. We have investigated how distinct promoter classes shape initiation differently by exploring their TSS characteristics (distances to recognizable promoter elements and broadness of TSS distributions) and the sequence contexts of promoter regions. We also examine functional requirements for proposed core promoter elements of TATA-less promoters to better understand how this class of promoter directs Pol II initiation.

303C. CK2-dependent Regulation of Ifh1 Transcription Factor Involved in the Expression of Ribosomal Protein Genes. ***Myung Sup Kim***, *Ji-Sook Hahn.* School of Chemical and Biological Engineering, Seoul National University.
In Saccharomyces cerevisiae, the regulation of ribosomal protein (RP) genes is mediated by Fhl1 and its co-activator Ifh1 and co-repressor Crf1. Fhl1 interacts with Ifh1 under normal conditions, but upon nutrient starvation, inhibition of TORC1 (target of rapamycin complex1) signaling pathway leads to the nuclear localization of Crf1 which binds to Fhl1, replacing Ifh1. However, the regulatory mechanism for the nutrient-dependent interaction between Fhl1 and Ifh1 is not understood. Here, we demonstrate that CK2-dependent phosphorylation of Ifh1 and Crf1 in the FHB domain of each protein is the key to their interactions with the FHA domain of Fhl1. Notably, ifh1 cells expressing unphosphorylatable Ifh1 mutant showed reduced levels of RP transcripts compared with cells expressing wild type Ifh1. Finally, we show that overexpression of catalytic subunits of CK2 results in increased RP gene expressions. Taken together, these findings suggest the mechanisms by which the recruitment of Ifh1 and Crf1 at RP gene promoters governs the transcription of RP genes.

304A. Effect of Modulating the Level of the Seventh Largest Subunit of RNA Polymerase II in Schizosaccharomyces pombe. ***Deepak Kumar***, *Nimisha Sharma.* University School of Biotechnology, G.G.S Indraprastha University, Sec.16C, Dwarka, New Delhi, India-110078.
Eukaryotic RNA polymerase II is a twelve-subunit enzyme that plays an important role in the control of gene expression in response to both external and internal stimuli. Its seventh largest subunit, Rpb7p, is highly conserved from yeast to humans and associates with the fourth largest subunit, Rpb4p, to form a heterodimer. Deletion of rpb7+ is lethal for the yeast cells. The function(s) of the Rpb7p subunit and the Rpb4p/Rpb7p complex are still poorly understood in Schizosaccharomyces pombe and other higher eukaryotic organisms. In this work, we have initiated experiments to elucidate the function(s) of the Rpb7p subunit in S. pombe. Towards this goal, we expressed rpb7+ under the control of the

thiamine-regulated nmt promoter and investigated its role in regulating multiple phenotypes in S. pombe. It was observed that under optimum conditions cells expressing reduced levels of Rpb7p in the presence of thiamine exhibited slow growth. However, no defect in growth was observed under a variety of stress conditions tested, including heat stress, osmotic stress, oxidative stress as well as survival during stationary phase. Interestingly, cell survival under nitrogen-limiting conditions was sensitive to Rpb7 levels. Our results further demonstrate that S. pombe cells expressing reduced levels of rpb7+ were elongated and showed cell separation defects. We also show that reduced rpb7+ levels resulted in a specific down-regulation of cell separation genes.

305B. DNA Replication Checkpoint Regulation of Cell-Cycle Transcription Dynamics. ***Adam R. Leman***[1], *Kevin A. McGoff*[2], *Xin Guo*[3], *John Harer*[2], *Steven B. Haase*[2]. 1) Biology, Duke University, Durham, NC; 2) Mathematics, Duke University, Durham, NC; 3) Statistical Sciences, Duke University, Durham, NC.
In *Saccharomyces cerevisiae*, a large number of genes are transcribed at specific intervals of the cell cycle. These genes oscillate in successive waves of transcription that coordinate with cell-cycle progression. We are actively characterizing the transcription factor (TF) networks that direct the periodic expression of these oscillatory genes. While CDKs are a major coordinator of cell-cycle events, we have shown that the temporal program of cell-cycle transcription is still largely intact in cells lacking CDK activities. Although cell cycle transcription can continue when cells are arrested by the depletion of CDK activities, we have recently demonstrated that the entire cell-cycle transcription program is halted during activated DNA Replication or Spindle checkpoint in *S. cerevisiae*. These findings suggest that checkpoint signaling pathways ensure that transcriptional state to is coupled to cell-cycle arrest. It has previously been shown that DNA replication checkpoint kinases target many cell-cycle transcription factors upon activation. However, it has yet to be determined what checkpoint kinase targets are the functional regulators of cell-cycle transcription during a checkpoint. We hypothesize that a subset of these checkpoint kinases are responsible for regulating specific TFs within the cell-cycle transcription network in order to halt the entire program. We ablated the primary downstream DNA replication checkpoint kinases, Rad53 and Dun1, then analyzed the global transcriptional response through time during a checkpoint response with and without these checkpoint kinases. In the absence of functional Rad53, we observed a large-scale restoration of cell-cycle transcription oscillations, even while cells were maintained in checkpoint arrest. To address which network TFs are regulated by Rad53 kinase during checkpoint activation, we applied a novel quantitative approach to compare transcription network dynamics in the presence and absence of functional checkpoint kinase activity. We have identified multiple functional targets of checkpoint kinases that impinge on cell-cycle transcription during a DNA replication checkpoint. Therefore, Rad53 is a central signaling component of the DNA replication checkpoint for halting cell-cycle transcription dynamics.

306C. The thiamine signal transduction pathway in *Candida glabrata*. ***Sarah Grace Leone***, *Nicholas Attanasio, Michael Peel, Christine Iosue, Dennis Wykoff.* Biology, Villanova University, Villanova, PA.
Thiamine pyrophosphate (TPP) is an essential cofactor for metabolic enzymes including pyruvate decarboxylase and pyruvate dehydrogenase. During thiamine starvation, *Saccharomyces cerevisiae* and *Candida glabrata* activate the thiamine signal transduction (THI) pathway to increase intracellular TPP concentrations. *S. cerevisiae* and *C. glabrata* utilize the thiamine transport pathway and the thiamine biosynthesis pathway to regulate thiamine levels. Induction of thiamine biosynthetic genes is regulated by Thi3, a TPP sensor and a transcriptional regulator. In the absence of thiamine, *S. cerevisiae* Thi3 associates transcription factors, Thi2 and Pdc2, to form an active transcriptional complex. In high thiamine, TPP binds to Thi3 preventing complex formation. The mechanism of gene regulation is well characterized in *S. cerevisiae*; however it is unclear how *C. glabrata* regulates THI biosynthetic genes especially since it lacks *THI2*. Thi2 is a necessary transcription factor for the induction of biosynthetic genes in *S. cerevisiae* and many other closely related yeast species. Through a series of complementation assays, we conclude that *C. glabrata* behaves similarly to a *S. cerevisiae thi2* strain and compensates for this gene loss by overexpressing *CgTHI3*. In addition, we identified THI regulatory elements in the promoters of three different thiamine biosynthetic genes. Results from the promoter truncation experiments have identified a 100bp region within these promoters that confer regulation by Thi3 in *C. glabrata*.
After conducting a comparative genomic study between *C. glabrata* and *S. cerevisiae* we discovered that C. glabrata lacks a family of genes (*THI5, THI11,THI12, THI13*) responsible for the biosynthesis of HMP-P, a subunit of thiamine. Therefore, we characterized expression levels of THI genes in *S. cerevisiae* and *C. glabrata* to determine which transport and biosynthesis pathway genes were up-regulated during thiamine starvation. Our qRT-PCR data show that *C. glabrata* highly induces the thiamine transporter *THI10*, while *S. cerevisiae* highly induces a HET-P biosynthetic gene *THI4*. These data suggest that *C. glabrata* utilizes the transport pathway while *S. cerevisiae* utilizes the biosynthesis pathway to increase intracellular thiamine levels. Determining the evolutionary differences between thiamine signal transduction pathways in yeast may uncover novel drug targets for antifungal treatments.

307A. Transcriptional Regulation of *HAP4* by the Mediator Complex and Adenine Levels in *Saccharomyces cerevisiae*. *Chad Bush, Denise Capps, Mengying Chiang, Tammy Pracheil,* ***Zhengchang Liu***. Dept. of Biological Sciences,

University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148.
The Hap2-5 complex is a major transcriptional activator of mitochondrial biogenesis, controlling the expression of many genes involved in respiratory metabolism. Hap4 is the regulatory subunit and its level determines the activity of the complex. *HAP4* expression is greatly induced when cells switch from fermentative to respiratory growth. However, how *HAP4* expression is regulated is still largely unknown. Using ethyl methanesulfonate (EMS) mutagenesis, we generated 38 mutants with increased *HAP4* expression when grown under high glucose conditions. We found that *HAP4* expression was affected by mutations to *SIN4*, *CYC8*, *SRB8*, and *ADE2*, components of two separate pathways: transcriptional regulation by the Mediator complex and de novo purine nucleotide biosynthesis. Sin4 and Srb8 are part of the RNA polymerase II Mediator complex which consists of 25 Med proteins. The Mediator complex associates with core polymerase subunits to form the RNA polymerase II holoenzyme which contributes to both positive and negative transcriptional regulation. We found that deletion mutations in 12 out of the 14 non-essential *MED* genes lead to increased *HAP4* expression to varying degrees. Cyc8 is a component of the Cyc8-Tup1 corepressor, which has been reported to genetically interact with components of the Mediator complex in mediating target gene expression. Ade2 is a metabolic enzyme in the de novo purine nucleotide biosynthesis pathway. *ade2* mutations from our genetic screen result in adenine deficiency and a consequential induction of *HAP4* expression. While it is unclear how an adenine deficiency would lead to increased *HAP4* expression, the discovery that mutations to the Mediator complex components affect *HAP4* expression suggests that the Mediator complex may regulate mitochondrial biogenesis though its effect on *HAP4* expression. Our data provide insights into the mechanisms underlying the regulation of *HAP4* expression and mitochondrial biogenesis in yeast.

308B. Loss of Ubp3 increases Silencing, decreases Unequal Recombination in rDNA, and shortens the Replicative Life Span in Saccharomyces cerevisiae. ***David Oling***, *Rehan Masoom, Kristian Kvint.* Chemistry and Molecular Biology, Medicinaregatan 9C, 41390, University Gothenburg, Sweden.
Ubp3 is a conserved ubiquitin protease that acts as an anti-silencing factor in MAT and telomeric regions. Here, we also show that *ubp3* mutants display increased silencing in rDNA. Consistent with this, RNAPII occupancy is lower in cells lacking Ubp3 than in wild type cells in all heterochromatic regions. Moreover, in a *ubp3* mutant, unequal recombination in rDNA is highly suppressed. We present genetic evidence that this effect on rDNA recombination, but not silencing, is entirely dependent on the silencing factor Sir2. Also, *ubp3 sir2* mutants age prematurely at the same rate as *sir2* mutants. Thus, our data suggest that recombination negatively influences replicative life span more so than silencing. However, in *ubp3* mutants recombination is not a prerequisite for aging since cells lacking Ubp3 have a shorter life span than isogenic wild type cells. We discuss the data in view of different models on how silencing and unequal recombination affect replicative life span and the role of Ubp3 in these processes.

309C. Phenotypic landscape of the conserved and essential RNA Polymerase II trigger loop: a high-throughput structure-function analysis. ***Chenxi Qiu***, *Olivia Erinne, Ping Cui, Kenny Lam, Sabareesh Babu, Huiyan Jin, Alvin Tang, Nandhini Mutukrishnan, Craig Kaplan.* Biochemistry&Biophysics, Texas A&M University, College Station,TX.
Transcription regulation is the first step of controlling gene expression and is critical for cellular function. The trigger loop (TL) is a highly conserved domain in the large subunit (Rpo21/Rpb1) of RNA polymerase II with roles in substrate selection, catalysis, and enzyme translocation during transcription. In order to functionally dissect the Pol II TL at an individual amino acid resolution in Saccharomyces cerevisiae, we are developing a deep-sequencing based methodology for quantitatively phenotyping a very large set of TL variants in parallel. Using this method, we have assessed almost every possible single amino acid substitution mutant along with a subset of doubly-substituted TL mutants for a number of growth phenotypes. Transcription-related in vivo phenotypes and the ability to uncover the intra-TL genetic interaction landscape will allow insights into Pol II active site function and generate a highly useful functional resource complementary to mechanistic structural and computational studies on Pol II. Our recent progress on this project will also be discussed.

310A. Analysis of ncDNA transcription for roles in regulating gene expression. ***Elizabeth A Raupach***[1], *Joseph Martens*[2]. 1) Biological Sciences, University of Pittsburgh, PIttsburgh, PA; 2) Biology, Hamilton College, Clinton, NY.
Transcription of non-coding DNA (ncDNA) is widespread in eukaryotes and plays important regulatory roles. Previous studies have elucidated one such regulatory mechanism at the *S. cerevisiae* gene *SER3*. The act of transcribing *SRG1*, a non-coding RNA (ncRNA), across the *SER3* promoter positions nucleosomes over the *SER3* upstream activating sequences, which serve as a physical barrier to prevent transcription of *SER3*. The pervasiveness of non-coding transcription suggests that this and other regulatory mechanisms mediated by non-coding transcription may exist throughout the genome. To explore this possibility, I selected six candidate yeast genes expressing unstable ncRNAs over their promoters and analyzed the effects of disrupting intergenic transcription on open reading frame transcript expression. Through this unbiased approach, we identified a previously unknown mechanism of transcription regulation at the *ECM3* gene. In contrast to the mechanism of *SER3* regulation, intergenic transcription seems to activate *ECM3* expression. Further analysis has identified roles for the Paf1 complex in *ECM3* activation through the methylation of histone H3K4.

Additionally, a longer intergenic transcript is present in the absence of Paf1. Although this transcript awaits further characterization, it may arise from a termination defect and could potentially provide a spatial and/or temporal dimension to *ECM3* regulation. Thus, *ECM3* is an interesting model gene for elucidation of a novel regulatory mechanism mediated by non-coding transcription.

311B. Functional analysis of gene expression within the *Lachancea kluyveri* species. *Christian Brion, David Pflieger,* ***Joseph Schacherer***. Department of Genetics and Genomics, University of Strasbourg, Strasbourg, France.
Genetic and phenotypic variations are the two aspects of intra-specific diversity. Changes in gene expression are a proxy, which can be used to assess the link between genetic and phenotypic variation. Among isolates of *Saccharomyces cerevisiae*, expression has been widely studied in various growth conditions. However, studies concerning this model organism are not sufficient to elucidate the underlying causes of phenotypic variation in all yeast, thus it is necessary to expand this type of analysis to additional species. We studied the expression of 24 recently sequenced strains of the preduplicated yeast *Lachancea kluyveri*. Using RNAseq, we analyzed expression levels in the strains at mid-log phase in non-stressful conditions. No correlation was observed between phylogenetic clusters based on nuclear divergence and those from expression profiles. Using a clustering analysis, we grouped genes that had the same expression pattern across the population. As expected, functional enrichment was observed for each of the following clusters: ribosome biogenesis, aerobic respiration or glycolysis. The genome of *L. kluyveri* was annotated based on the sequence similarity compared to the homologous gene found in S. cerevisiae. As genes that display the same expression pattern are likely involved in the same biological process accurate gene functional annotation could be completed. For example, the gene *SAKL0H23584g*, with no known sequence similarities, displays the same expression pattern as the *STE2* homolog which suggests it possibly plays a role in determining mating type and pheromone response. We developed a pipeline to complete this analysis automatically at the whole genome scale. The genes implicated in specific phenotypes, such as growth rate, were also identified by correlation. Expression analysis is a powerful tool to expand our knowledge about new species. We have described intra-specific transcriptomic variation and improved genome functional annotation of the *L. kluyveri* species. The next step is to expand the functional analysis of these genomes by studying expression response to environmental changes and ultimately obtain a better overall picture of the molecular basis for phenotypic variation based on transcriptomic analyses.

312C. Functional analysis of stress regulated non-coding RNAs in budding yeast. ***Amanda N. Scholes****, Tara N. Stuecker, Jeffery A. Lewis.* Biological Sciences, University of Arkansas, Fayetteville, AR.
Recent studies ranging from humans to yeast have shown pervasive transcription of non-coding regions. While the vast majority of these non-coding (nc) RNAs remain uncharacterized, there is strong evidence that certain ncRNAs have important regulatory functions. However, systematic characterization of ncRNAs and their functions is a major challenge. We hypothesize that functional ncRNAs will only be expressed under certain conditions. Thus, identifying ncRNAs that respond to environmental perturbation may provide unique insight into their biological roles. We used tiling microarray data from our lab and others, and identified several hundred ncRNAs that respond to diverse environmental stressors. We chose to focus on ncRNAs that were antisense and overlapping with coding genes (sense/antisense or S/AS pairs). S/AS pairs are extremely prevalent (64% of all coding genes have at least one anti-sense ncRNA), and S/AS UTRs of coding genes have been implicated in transcriptional interference. Our data analysis found a widespread negative correlation between the expression of coding regions and their corresponding anti-sense ncRNAs (31% of S/AS pairs are anti-correlated under at least one condition). We are currently using molecular genetics to test whether expression of the ncRNAs affects coding gene expression, by inserting transcriptional terminators into representative ncRNAs. These studies are providing new insight into the cellular functions and regulatory mechanisms of ncRNAs.

313A. FACS-based genetic screen in *S. cerevisiae* identifies genes involved in the alpha-factor response. ***Anna Sliva***[1,2], *Zheng Kuang*[1,2], *Jef Boeke*[1,2]. 1) High Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore, MD 21205; 2) New York University Langone Medical Center, New York, NY 10016.
Dynamic biological processes have proven challenging to study. The mating pathway in yeast is a well-understood dynamic system, making it a good model for the development of new screening methods. Upon exposure to pheromone from the opposite mating type, haploid cells upregulate the expression of many genes that enable formation of *MAT***a**/ diploids. One of the genes that increase in expression upon induction with alpha factor is *FUS1*, a gene involved in cell fusion. Wild type cells with an intact mating pathway express *FUS1* at basal levels and have increased *FUS1* expression upon pheromone induction. Cells defective in the pathway do not have even basal *FUS1* levels, or express basal levels of *FUS1* but fail to increase *FUS1* expression upon alpha factor induction. A reporter construct containing the *FUS1* promoter driving GFP expression was made and transformed into a pool of *MAT***a** haploid yeast knockout (YKO) library mutants. The pool was induced with alpha-factor and sorted into three different populations based on GFP expression by FACS: GFP negative, basal GFP, and GFP positive populations. The barcodes of the sorted cells were PCR amplified and sequenced to identify the mutants present in each population. The sequencing results showed a significant enrichment of

expected mutants in the GFP negative (*ste4, ste5,* and *ste11*) and GFP basal (*ste2* and *ste20*) populations. Additionally, three of the top hits that were highly enriched in the GFP negative and GFP basal populations not known to be involved in the mating pathway, *rps12, rsm25,* and *bre1,* were retested individually and have shown recapitulation of the mutant *FUS1*-GFP expression phenotype.

314B. Adaptive evolution of transcription and translation in pathogenic yeast. ***Xuepeng Sun***[1,2]*, Zhe Wang*[1]*, Zhenglong Gu*[1]. 1) Division of nutritional sciences, Ithaca, NY; 2) College of Agriculture and Biotechnology.
A great deal of research has focused on the genetic variation contributed to pathogenicity. Some has revealed the dispensable chromosome (Ma, et al., 2010), specific genes clusters (Kamper, et al., 2006), pathogenicity related gene family expansion (Butler, et al., 2009), and gene copy number variation (Moran, et al., 2011) as the virulence determinant in several fungal pathogens. However, quite few attentions were paid on the evolution of gene transcription and translation relating to the pathogenicity. In this study, we compared the gene expression and translation between lab strain and clinic strain of S. cerevisiae, as well as S. paradoxus as outgroup. We found that, (1) the evolutionary rate of gene transcription and translation is much faster than gene sequences; (2) totally, 2,436 genes showed differences between lab strain and clinic strain, of which, more than one-third differential expressed genes in clinic strain were lineage specific, and were strong related to mitochondrial translation and protein transporting processes; (3) ca. 45 percent genes changed in translation efficiency were lineage specific, and these genes were strong enriched in intracellular transport and mRNA metabolic processes; (4) as the evidence for adaptive evolution, we found several protein complex, which the selective sweep were observed around the coding regions, were directional regulated, suggesting the potential roles in pathogenicity.

315C. PRS gene family - a means of linking primary metabolism and cell signalling. ***Eziuche Amadike Ugbogu***, *Lilian Marry Schweizer, Michael Schweizer.* Biochemistry, Heriot-watt University, Edinburgh, United Kingdom.
PRPP (5-phosphoribosyl-1()-pyrophosphate) is a key metabolite that plays a central role in many life processes, such as in the de novo and salvage biosyntheses of purine, pyrimidine and pyridine nucleotides. The genome of the model eukaryote Saccharomyces cerevisiae contains five highly homologous paralogous genes, designated PRS1-PRS5, encoding polypeptides which exist in the cell as two complexes, one of which is a heterodimer, Prs1/Prs3 and the other, a heterotrimer, Prs2/Prs4/Prs5. Physical evidence for the genetically-defined Prs1/Prs3 complex was obtained showing that in the absence of Prs3, Prs1 is unstable. In addition to supplying the cell with PRPP, Prs polypeptides are required for the maintenance of cell wall integrity (CWI) since the non-homologous region of Prs1 (NHR1-1) interacts with Slt2, a component of the CWI pathway. Prs5 is unusual in that it is triply phosphorylated and contains two NHRs. Mutation of the three postulated Prs5 phosphorylation sites and the neighbouring region also influences CWI as demonstrated by altered Rlm1 expression and the pattern of Slt2 phosphorylation. It is hypothesized that Prs1 and Prs5 may have arisen from the prototype Prs-encoding genes, Prs2, Prs3 and Prs4, by duplication followed by acquisition of NHRs thereby linking primary metabolism with CWI signalling.

316A. S. cerevisae rRNA Synthesis by RNA Polymerase (Pol) II in Response to Nitrogen Deprivation. ***Arjuna Rao Vallabhaneni****, Merita Kabashi, Kushal Bhatt, Heather Conrad-Webb.* Biology, Texas Woman's University, Denton, TX.
The synthesis of rRNA is highly regulated since a large portion of the cells resources are devoted to the synthesis of ribosomes. During stress such as mitochondrial dysfunction, nitrogen deprivation and osmotic shock, Pol II synthesizes a significant percentage of rRNA using a cryptic pol II rDNA promoter. Pol II mediated rRNA synthesis may be a universal stress response since higher eukaryotes have conserved an overlapping pol I/pol II rDNA promoter (1,2,3). To dissect the activation of pol II rRNA synthesis during nitrogen deprivation, members of the TOR pathway and general stress response pathways were examined for pol II rRNA synthesis using rDNA-lacZ reporter genes. The TOR pathway is the master regulator of cellular responses to available nutrients, regulating all three RNA polymerases. In the absence of upstream members of TOR pathway, such as Tor1 and Sch9, there is increase in Pol II rRNA synthesis even in the absence of stress consistent with their role in repressing stress response genes. In addition, the absence of downstream effectors Rim15, and general stress transcription factors, Msn2/4, results in decreased Pol II rRNA suggesting that the TOR pathway regulates Pol II rRNA synthesis via Msn2/4. The HDAC Rpd3 and mediator complex that are required during multiple stress responses are also required for pol II rRNA synthesis (4). This suggests that their role in modulating chromatin structure in response to stress facilitates the switch from pol I to pol II for rRNA synthesis. Chromatin immunoprecipitation experiments are being carried out to determine if Msn2/4 directly activates Pol II rRNA synthesis. These studies will help understand the mechanism of Pol II rRNA synthesis and its role in stress adaptation. 1.Pikkard, C.S (1994). Proc.Natl Acad Sci USA 91, 464-468 2.Smale, S.T., and Tjian, R (1985). Mol.Cell.Biol. 5, 352-362 3.Sollner-Webb, B and Mougey, E.B. (1991) Trends Biochem. Sci. 16, 58-62 4.Alejandro-Osorio, A.L et al., (2009) Genome Biology 10 (5), R57.2.

317B. Systematic analysis of RNA polymerase III and TFIIIB phosphoregulation in *Saccharomyces cerevisiae. Jaehoon Lee, Robyn D. Moir,* ***Ian M. Willis***. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY.

The production of ribosomes and tRNAs for protein synthesis has a high energetic cost and is under tight posttranslational control to ensure that the level of RNA synthesis is balanced with nutrient availability and the prevailing environmental conditions. In the RNA polymerase (pol) III system in yeast, nutrients and stress affect transcription through a bifurcated signaling pathway in which protein kinase A (PKA) and TORC1 activity directly or indirectly (through downstream kinases) alters the phosphorylation state and function of the Maf1 repressor and a TFIIF-like subunit of the polymerase, Rpc53. However, numerous lines of evidence suggest a more complex regulatory network involving the phosphoregulation of other pol III components. To address this issue, we systematically examined all 17 subunits of pol III along with the three subunits of TFIIIB for evidence of differential phosphorylation in response to inhibition of TORC1. A relatively high stoichiometry of phosphorylation was observed for several of these proteins and the Bdp1subunit of TFIIIB was found to be differentially phosphorylated. The data show that Bdp1 is phosphorylated on four major sites during exponential growth by at least two kinases and that the protein is variably dephosphorylated under repressing conditions. The phosphorylation of Bdp1 at these sites opposes Maf1-mediated repression since a Bdp1 4SA mutant is hyper-repressible. A regulatory model that incorporates these new findings will be presented.

318C. Defining the transcriptome of *Saccharomyces cerevisiae*. *Janos Demeter, Paul Lloyd,* ***Edith Wong****, J. Michael Cherry.* Department of Genetics, Stanford University, Palo Alto, CA.
The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in a single cell or a population of cells. Defining the complete transcriptome of the budding yeast, a single celled eukaryotic organism, should be an achievable goal with the use of high-throughput genomic technologies (e.g. tiling microarrays, next generation sequencing). Indeed, numerous publications have addressed this question and made their datasets publicly available. As a scientific database that provides researchers with high-quality curated data, the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) set out to collect a representative collection of these high quality and frequently cited datasets with the goal of compiling them into a complete transcriptome in yeast - a longstanding request of our users. Integration of these datasets was more complicated than anticipated for a number of reasons. First, exact matches between the datasets did not even remotely cover the genome completely. One major issue is the various technologies producing different kinds of results. For example, some approaches give only the 5'-end, others only the 3'-end and yet others both ends of the messages. Our integrative approach raises the question whether to define transcription start and end points or entire transcripts. Another issue to be resolved is the detection of low abundance transcripts and their functional significance. We present the results of our first attempt to define a transcriptome for *S. cerevisiae.* Initially, we confined ourselves to a single common condition, wild type lab strains grown in rich medium. In the future, we will extend the analysis to a much wider list of conditions.

319A. Dissection of the PHO pathway in *S. pombe* using epistasis and the alternate repressor adenine. *Molly S. Estill, Christine L. Iosue,* ***Dennis D. Wykoff***. Dept Biol, Villanova Univ, Villanova, PA.
Proper nutrient sensing is required for cell survival. In *Saccharomyces cerevisiae,* cellular homeostasis of intracellular phosphate levels is maintained by the phosphate signal transduction pathway (PHO pathway), and is often assayed by the up-regulation of phosphatase activity. The PHO pathway of *Schizosaccharomyces pombe* also up-regulates phosphatase expression (encoded by $pho1^+$) in response to low extracellular phosphate levels, but the mechanism is much less characterized. We utilized an alternate repressor of $pho1^+$ expression (adenine supplementation) along with epistasis analysis to develop a model of how *S. pombe* PHO pathway components interact and we describe a putative mechanism behind the cross-regulation of the *S. pombe* PHO pathway by adenine starvation.
We previously identified Pho7, a zinc-finger transcription factor, and confirmed that it is necessary for both the adenine and phosphate starvation-mediated increase in Pho1 activity. We hypothesized that regulators of $pho1^+$ during phosphate starvation would act during adenine starvation and analyzed Pho1 activity in *S. pombe* PHO pathway deletion mutants during adenine starvation. Most mutants also had a phosphatase defect in adenine starvation. When comparing adenine starvation relative to phosphate starvation, there are some differences in the degree to which individual mutants regulate the two responses, suggesting cross-regulation as well as divergent regulation. Through epistasis studies of the *S. pombe* gene deletions, we were able to determine the interactions between these positive and negative regulators. We identified two positive regulatory arms and one repressive arm of the pathway. PKA activation is a positive regulator of Pho1 under both environmental conditions, and this signaling is critical for transducing adenine concentrations to the cell. The synthesis of IP_7 also appears critical for the induction of Pho1 activity during adenine starvation, but IP_7 is not critical during phosphate starvation, which is different from S. cerevisiae. Finally, Csk1 is critical for repression of $pho1^+$ expression during both starvation conditions, but appears more important for repression with phosphate supplementation. We believe all of these regulatory arms converge on Pho7 phosphorylation status and regulate its ability to increase transcription of $pho1^+$ and other genes.

320B. Accumulation of a threonine biosynthetic intermediate attenuates general amino acid control by inducing degradation of promoter-bound Gcn4. ***FNU Yashpal****, Hongfang Qiu, Alan G. Hinnebusch.* Laboratory of Gene Regulation

and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892.
Transcriptional activator Gcn4 maintains amino acid homeostasis in budding yeast by inducing multiple amino acid biosynthetic pathways in response to starvation for any amino acidthe general amino acid control (GAAC). Gcn4 abundance is tightly regulated by the interplay between an intricate translational control mechanism, which induces Gcn4 synthesis in starved cells, and a pathway of phosphorylation and ubiquitylation that mediates its rapid degradation by the proteasome. Mutations impairing Gcn4 function confer sensitivity to inhibitors of amino acid biosynthesis, including sulfometuron (SM) that blocks isoleucine/valine production. Genetic screening revealed that deletion mutants lacking particular threonine biosynthetic genes exhibit sensitivity to SM, and we found that one such mutant (*hom6*) is defective for SM-induced transcriptional induction of Gcn4 target genes *HIS4* and *ARG1* and a Gcn4-dependent *lacZ* reporter. We demonstrated that the GAAC defect in *hom6* cells results from accumulation of the Hom6 substrate, aspartate semialdehyde (ASA), as it is suppressed by removing the first enzyme of the threonine pathway (Hom3) and exacerbated by eliminating feedback inhibition of Hom3 by threonine. Analysis of Gcn4 synthesis and turnover rates revealed that ASA accumulation accelerates the degradation of Gcn4, already an exceedingly unstable protein, and that promoter-bound Gcn4 molecules are targeted predominantly for rapid turnover. ASA-enhanced degradation of Gcn4 requires its phosphorylation by cyclin-dependent kinases (CDKs) Srb10 and Pho85. Although eliminating either Srb10 or Pho85 in *hom6* cells restores comparable levels of Gcn4 at a target promoter, recruitment of RNA Polymerase II and attendant transcription of target genes was restored only in the *hom6 pho85* double mutants, thus indicating that the specific activity of Gcn4 remains low in the cells lacking Srb10. These results unveil a division of labor between these two CDKs wherein Srb10 primarily targets inactive Gcn4 moleculespresumably damaged under conditions of ASA excess, and characterized by hypersumoylationwhile Pho85 clears mostly functional and hyposumoylated Gcn4 from the cell. The ability of ASA to inhibit transcriptional induction of threonine pathway enzymes by Gcn4, dampening ASA accumulation and its toxic effects on cell physiology, should be adaptive in the wild when yeast encounters natural antibiotics that target Hom6 enzymatic activity.

321C. Codon Context and Translation Efficiency in Yeast. ***Caitlin E. Gamble***[1], *Christina Brule*[2], *Stanley Fields*[1,3], *Elizabeth Grayhack*[2]. 1) Genome Sciences, University of Washington, Seattle, WA; 2) University of Rochester School of Medicine and Dentistry, Rochester, NY; 3) Howard Hughes Medical Institute.
Because of degeneracy in the genetic code, several codons can encode the same amino acid, yet the genomes of many organisms reflect a bias in the use of both individual codons and pairs of consecutive codons. Pair bias suggests that the translation machinery is sensitive to the properties of synonymous codons and their associated tRNAs. We had previously demonstrated in yeast that adjacent CGA-CGA codons are strong inhibitors of translation and are much more potent inhibitors than isolated CGA codons. Thus, we sought to identify pairs of non-identical codons with a strong inhibitory impact on yeast translation. We generated two libraries, each containing a three-codon insertion near the N-terminus of superfolder GFP. We performed fluorescence-activated cell sorting followed by high-throughput sequencing of the insertions to estimate the mean expression level of a total of 40,868 GFP variants. We found that for a small number of codon pairs, most variants containing that pair had low expression levels, whereas most variants exhibited high levels of expression. Overall, we identified 12 pairs with evidence of a general inhibitory impact. Reconstructed variants with these pairs had reduced GFP fluorescence levels relative to a synonymous construct. Additionally, in eight cases, overproduction of a tRNA or tRNA variant (in which the wobble base was mutated to exactly base pair with one codon in the inhibitory pair) substantially suppressed low GFP expression. Furthermore, the directionality of a pair was often central to inhibitory effects, and the surrounding nucleotide or amino acid contexts further modulated the degree of inhibition for each codon pair. Thus, we have experimentally identified a set of codon pairs that are likely to reduce translation efficiency due to non-optimal pair dynamics within the ribosome.

322A. Functional alnalysis of *CaMCA1* and *EDC3* in oxidative stress response and apoptotic cell death in *Candida albicans*. ***Jeong-Hoon Jeong***, *Jong-hwan Jung, Jinmi Kim.* Department of Microbiology and Molecular biology, Chungnam National University, Daejeon, South Korea.
Various stresses including weak acid, oxidative stress and UV irradiation are known to increase the accumulation of reactive oxygen species (ROS) and the number of apoptotic cells in yeast. The metacaspase CaMca1 plays a key role in the apoptotic cell death in *Candida albicans*. Edc3 is a decapping activator and functions as a scaffold protein of P-bodies. Recently we showed that the *edc3/edc3* deletion strain showed a filamentation defect and increased viabilities upon apoptotic stresses. Also, deletion of *EDC3* resulted in less intracellular ROS accumulation. The CaMca1 was down-regulated in *edc3/edc3* deletion strains compared to the wild-type strain. Over-expressed Mca1 suppressed the *edc3/edc3* phenotypes, including slower growth, increased resistance to oxidative stress, and less ROS accumulation during oxidative stress. These results suggest that the apoptosis related phenotypes of the *edc3/edc3* deletion strain are closely related to the decreased expression level of the CaMca1 in the *edc3/edc3* deletion cells. The oxidative stress related proteins, Cat1 and

Sod1, were up-regulated in *edc3/ edc3* deletion strains compared to the wild-type strain. We are currently interested in the post-transcriptional regulation mediated by Edc3 in apoptotic stresses.

323B. Roles of decapping activators in mRNA translation and P-body formation during mating. ***Daehee Jung***, *Yuseon Lee, Jinmi Kim.* Department of Microbiology and Molecular Biology, Chungnam National University, Daejeon, South Korea.
In the budding yeast *Saccharomyces cerevisiae*, two haploid cells of opposite mating type, a and , fuse to form a a/ diploid. The mating process is regulated by the mitogen-activated protein kinase which activates the mating-specific transcription factor Ste12. The Ste12 protein expression is affected by the deletion mutation in the *DHH1* gene, which encodes a DEAD-box helicase. Dhh1, Lsm1-7 complex and Pat1 are known to function as mRNA decapping activators and to be localized to processing bodies(P-bodies). P-bodies are increased upon -mating pheromone treatment and the Ste12 level is closely associated with P-body accumulation. To understand the translational mechanisms of Ste12 expression, mutational analysis of Dhh1 was carried out. The function of other decapping activators, Lsm1 and pat1, and translation initiation regulators, Eap1 and Caf20, were also investigated. In a previous study, we observed that the protein expression of Ste12, Gpa2 and Cln1 increased during yeast hyphal growth. Current interests are to analyze protein expressions of Gpa2 and Cln1 during mating and to find new target genes whose expressions are regulated by decapping activators, Dhh1, Lsm1 and Pat1.

324C. Evolutionary Divergence of the Stress Response at the Translational Level. ***Pieter J Spealman***, *Alan Shteyman, Charles McManus.* Biological Sciences, Carnegie Mellon University, PITTSBURGH, PA.
The ability to respond to the environment is essential to life and is critical for evolution. At the most fundamental level this response is a phenotypic change that allows the organism to adapt to different conditions. Previous work has shown that the transcriptional stress response is highly divergent between strains and species, suggesting that the stress response may not be as deeply conserved as was once believed (Tirosh et al., PNAS 2011, Kvitek et al. 2008). However, translational regulation may lead to more conserved stress responses in protein production than suggested by mRNA abundance measurements. To address these possibilities, we used ribosome profiling to compare transcriptional and translational stress responses to amino-acid starvation among members of the genus Saccharomyces. Response to this stressor has been well characterized at both the transcriptional and translational levels in S. cerevisiae (Ingolia et al., 2008). Research of translational regulation has become feasible with the recent introduction of ribosome profiling; a high-throughput sequencing technique that simultaneously measures transcript abundance, ribosome occupancy and translation efficiency. Our results do not support the hypothesis that divergence in stress response is buffered by either translational regulation. Upon amino-acid starvation mRNA abundance changed for 35% of genes in S. cerevisiae and 11% of S. paradoxus genes. 30% of the S. cerevisiae induced genes were also induced in S. paradoxus, while 73% of S. cerevisiae genes were repressed in both. This divergence is exacerbated at the level of translation where RPF abundance changed for 35% of genes in S. cerevisiae and 14% of S. paradoxus genes. 20% of the S. cerevisiae induced genes were also induced in S. paradoxus, while 65% of S. cerevisiae genes were repressed in both.

325A. Improving heterologous cytochrome P450 function in S. cerevisiae. ***Anita Emmerstorfer***[1], *Miriam Wimmer*[1], *Tamara Wriessnegger*[1], *Erich Leitner*[2], *Monika Müller*[3], *Iwona Kaluzna*[3], *Martin Schurmann*[3], *Daniel Mink*[3], *Guenther Zellnig*[4], *Harald Pichler*[1,5]. 1) ACIB GmbH, Petersgasse 14, 8010 Graz, Austria; 2) Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Stremayrgasse 9, 8010 Graz, Austria; 3) DSM Innovative Synthesis B.V., Urmonderbaan 22, 6167 RD Geleen, The Netherlands; 4) Institute of Plant Sciences, University of Graz, Schubertstrasse 51, 8010 Graz, Austria; 5) Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14/2, 8010 Graz, Austria.
Membrane-attached cytochrome P450 monooxygenases (CYPs) are versatile and industrially important enzymes, playing major roles in drug metabolism as well as production of fine chemicals, pharmaceutical compounds or flavors and fragrances. However, a number of limitations have restricted their use in industrial processes including narrow substrate specificity, the association of the enzymes with membranous structures, the co-expression of an appropriate cytochrome P450 reductase, the need for a complex system of cofactor regeneration and low turnover numbers. Therefore, approaches for improving overall CYP activity are versatile and highly complex. The aim of this study was the establishment of a genetically modified S. cerevisiae strain serving as expression platform for overally improved CYP activity. Displaying many cellular features and metabolic pathways similar or identical to those of higher eukaryotes, yeast cells are highly amenable to express recombinant CYPs. Furthermore, yeasts have the ability to synthesize diverse substrates like plant terpenes intracellularly upon expression of appropriate terpene synthases. This option helps to circumvent phase transfer issues of hydrophobic substances into yeast cells and allows the direct CYP-mediated conversion to produce terpenoids. Using both, resting and self-sufficient, terpene-producing cells, multiple approaches were evaluated for their potential to improve CYP activity like comparing different expression hosts, supporting enzyme stabilities and increasing intracellular cofactor and substrate supply.

326B. Mutants defective in stress granule formation exhibit a deregulated stress response. ***Elena Garre***[1], *Xiaoxue Yang*[2], *Yi Shen*[2], *Xinxin Hao*[1], *Daniel Krumlinde*[1], *Marija Cvijović*[3,4], *Christina Arens*[1], *Thomas Nyström*[1], *Beidong Liu*[1,2], *Per Sunnerhagen*[1]. 1) Department of Chemistry and Molecular Biology, University of Gothenburg, Göteborg, Sweden; 2) School of Life Science and Engineering, Harbin Institute of Technology, Harbin, China; 3) Department of Mathematical Sciences, Chalmers University of Technology, Göteborg, Sweden; 4) Department of Mathematical Sciences, University of Gothenburg, Göteborg, Sweden.
To reduce expression of gene products not required under stress conditions, eukaryotic cells form large and complex cytoplasmic aggregates of RNA and proteins (stress granules; SGs), where transcripts are kept translationally inert. The overall composition of SGs, as well as their assembly requirements and regulation through stress-activated signaling pathways remain largely unknown. We have performed a genome-wide screen of S. cerevisiae gene deletion mutants to identify modifiers of SGs formation under nutrient stress induced by 2-deoxyglucose (2 DG). Mutants identified in the primary screen as deficient or hyperproficient in SG formation were also tested for SG formation under other stress agents. Most of these mutants display a general defect in SG formation, whereas the defect in others is specific for certain stress types. It has previously been shown that SGs physically incorporate, and potentially dampen the activation of, signaling proteins. We wanted to test the possibility that the SG-forming ability under stress is mechanistically linked to the ability to raise and regulate various aspects of the cellular stress response. Importantly, we observe here that mutants with strongly reduced ability to form SGs upon stress are also deficient in their ability to modulate changes gene expression associated with the stress response. Expression analysis of some transcripts related with stress response performed with several mutants unable to form SGs showed that stress-activated transcripts are hyperinduced, whereas those down-regulated by stress are repressed even further than in the wild-type. This strongly indicates that not only is SG formation regulated by stress-activated signaling pathways, but SGs actively moderate the stress response at the transcript level.

327C. Securing autoselection in yeast ACNase killer toxin systems by mRNA fragmentation. ***Alene Kast***, *Friedhelm Meinhardt.* Institute of Molecular Microbiology and Biotechnology, University of Münster, Corrrensstr. 3, 48149 Münster, Germany.
Virus-like element (VLE) encoded yeast killer toxins act as ribonucleases specifically cleaving the target cells tRNA or rRNA, leading to a growth arrest of sensitive cells. The killer strains are immune against their own toxins. In the case of the tRNA attacking anticodon nuclease (ACNase) toxins immunity is conferred by a VLE encoded protein, which is specific for the accordant toxin. Expression of the immunity genes was until now solely possible from the cytoplasmic elements. We show that the mRNA is unstable in the nucleus as only transcript fragments can be detected by Northern analysis. The mRNA instability precludes the functional capture of the immunity gene by the yeast nucleus, which would disable autoselection of the VLEs.

328A. Gene copy number and colony morphology in wild yeast strains. ***Derek Wilkinson***[1], *Vratislav Stovicek*[3], *Libuse Vachova*[2], *Zdena Palkova*[1]. 1) Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Vinicna 5, 128 44 Prague 2, Czech Republic; 2) Institute of Microbiology of the ASCR, v.v.i., Videska 1083, 142 20 Prague 4, Czech Republic; 3) The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kogle Alle 6, 2970 Hørsholm, Denmark.
Wild strains of yeast form complex, structured colonies when compared with the simpler, smooth colonies formed by domesticated strains. However, the growth of a domesticated strain under adverse conditions may result in a reversion to feral sub clones forming colonies with wild type-like morphology. We derived the domesticated strain BR-S, from a wild-type strain BR-F and subsequently derived the feral strain BR-RF from BR-S [1]. Genome-wide transcriptomic analysis of colonies of the strains revealed that the expression of genes clustered in specific regions of a small number of chromosomes is upregulated in the feral (BR-RF) strain, compared with both the wild (BR-F) and domesticated (BR-S) strains. We then used real time q-PCR to measure the copy numbers of upregulated genes in the left arm of chromosome XV relative to those of non-upregulated genes in the right arm of the same chromosome. We found that the copy numbers of three genes YOL110W, YOL060C and YOL038W, relative to three reference genes YOR194C, YOR250C and YOR288C were higher in strain BR-RF than in strain BR-S. YOL110W (SHR5) encodes a palmitoyltransferase subunit, regulating Ras2p localisation to the plasma membrane, YOL060C (MAM3) encodes a protein with a role in mitochondrial morphology and YOL038W (PRE6) encodes an essential alpha subunit of the core proteasome. Increased copy numbers of genes within specific chromosomal regions indicate large duplications in the genome which occur during the reversion process from domesticated to feral cell type under stress conditions. However, such rearrangements in BR-RF are unrelated to the structured colony phenotype. [1] Stovicek et al (2014) BMC Genomics 15: 136. doi: 10.1186/1471-2164-15-136.

329B. Chaperonins enabled functional expression of bacterial xylose isomerases in yeast. ***Jianjun Yang***[1], *Min Qi*[1], *Kristen Kelly*[1], *Sarah Rush*[2], *Luan Tao*[2], *Rick Ye*[1], *Paul Viitanen*[2], *William Hitz*[2]. 1) Biotechnology, DuPont Central Research and Development, Experimental Station, Wilmington, DE; 2) DuPont Industrial Biosciences, Experimental Station,

Wilmington, DE.
Hydrolysate prepared from cellulosic biomass contains two major sugars: glucose and xylose. Use of these sugars from the hydrolysate for fermentative production of fuel ethanol is desirable, as this is a readily renewable resource that does not compete with the food supply. Saccharomyces cerevisiae is a traditional ethanologen for ethanol fermentation, using hexoses obtained from grains or mash as the carbohydrate source. Because it is not naturally capable of metabolizing xylose, Saccharomyces cerevisiae can only be used in fermentation of biomass hydrolysate after a xylose assimilation pathway has been established. Many bacteria are able to utilize xylose through a pathway consisting of xylose isomerase and xylulose kinase. However, when this pathway was introduced into Saccharomyces cerevisia, success in functional expression of heterologous bacterial xylose isomerase has been limited. This report will describe our approach to co-express bacterial chaperonins and xylose isomerase. It demonstrates that E. coli GroES/GroEL enabled functional expression of bacterial xylose isomerase so as to establish a bacterial type xylose assimilation pathway in Saccharomyces cerevisiae.

330C. Dissecting the mechanism of gene-dosage response in naturally aneuploid, wild isolates of *Saccharomyces cerevisiae*. ***Mun Hong Yong***[1], *James Hose*[1], *Audrey Gasch*[1,2]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI.
Lab yeast strains are extremely sensitive to aneuploidy, the condition of having an abnormal number of chromosomes. Chromosomal duplication in the lab strain was previously shown to increase the expression of most amplified genes, which may disrupt balanced expression across the transcriptome and thus slow down growth rate. However, we have observed that many wild yeast strains are naturally aneuploid and show little growth disadvantage. We also found that expression of most amplified genes in wild strains does not increase proportionately to DNA copy. This suggests that wild yeast strains have either adapted to aneuploidy by heritably reducing expression of amplified genes or that they actively reduce expression of amplified genes in proportion to gene copy number. To test this, we generated isogenic strain panels in which diploid cells carry either two, three, or four copies of amplified chromosomes. We found that many genes display a dosage response - the expression does not increase proportionately with gene copy across the strain panel. This group of genes was enriched for mitochondrial genes. To test if these genes display feedback regulation, we cloned genes of interest onto a plasmid and transformed both lab and wild yeast strains with the plasmid to create gene duplication in the absence of aneuploidy. We found that the expressions of most cloned mitochondrial genes increased two-fold when an extra copy was introduced on a plasmid, indicating that there is no active dosage response when the gene is duplicated on a plasmid. This suggested that their reduced expression in aneuploid strains is either a general response to aneuploidy or is a response to amplified repressors encoded on the affected chromosome. To test this, we derived a wild strain that is naturally aneuploid for Chr XII, but in which the majority of the extra Chr XII was deleted, and measured the gene expression of the remaining duplicated genes. We found that most mitochondrial genes that remained amplified in the derived strain showed proportionately higher expression when the right arm of Chr XII was deleted compared to when the entire chromosome was amplified. Expression of these genes was not altered in response to other aneuploid chromosomes, indicating that reduced expression from amplified mitochondrial genes is not a generic aneuploidy response. This instead suggests that Chr XII carries specific repressors of the genes in question that maintain mitochondrial gene expression despite Chr XII aneuploidy. The current ongoing work is to test for the existence of such repressors on Chr XII.

331A. Rerouting resources from cell size to population growth drives evolution of cancer drug resistance in yeast. ***E Alonso-Perez***[1], *N Srinivas*[1], *M Kakavandi*[1], *K Ludwig*[1], *I Jonassen*[2], *SW Omholt*[3], *P Sunnerhagen*[1], *J Warringer*[1]. 1) Department of Chemistry & Molecular Biology, University of Gothenburg. Gothenburg, Sweden; 2) Uni Computing, Uni Research AS, Bergen, Norway ; Department of Informatics, University of Bergen, Bergen, Norway; 3) Center for Integrative Genetics (CIGENE), Norwegian University of Life Sciences (UMB), Ås, Norway.
Drug resistance emerging in tumour cells through adaptive evolution is a major obstacle to establishing efficient chemotherapy regimes. Tracing fitness contributions of individual mutations under cancer drug exposure and understanding their mechanistic basis are formidable challenges. Using yeast populations as tumour proxies, we followed evolution of cancer drug resistance under four chemotherapy regimes: hydroxyurea, rapamycin, cisplatin and doxorubicin exposure. Surprisingly, under two of these regimes, adaptive evolution were driven by more efficient utilization of energy and carbon rather than by increases in population net growth rate. Focusing on the evolution of hydroxyurea tolerance, we associcated growth efficiency increases to striking changes in cell morphology. Hydroxyurea exposure forced channelization of resources into swollen and pseudohyphae like cell structures. Adapted populations however retained normal cell size and shape, allowing channelization of excess energy and carbon into next generation biomass. We traced this rerouting of resources to clones containing mutations in the cell cycle regulator Whi2 known to control G1 to S transitions. Uniformly, clones maintained normal expression of the G1 cyclins Cln1 and Cln2, despite hydroxyurea exposure forcing a 100 fold increase in early stationary phase expression of these genes in non-adapted populations. This suggests a model where retention of a normal cell cycle progression under hydroxyurea exposure, via Whi2 regulation on

the G1 to S transition, frees up resources from grossly abnormal cell size growth to normal population expansion and highlights a novel mechanism of cancer drug adaptive evolution.

332B. Inferring Genetic Networks through Evolutionary Signatures. *Zelia Ferreira[1], Jennifer Walker[1], Allyson O'Donnell[2],* ***Nathan Clark***[1]. 1) Computational and Systems Biology, University of Pittsburgh, PA; 2) Department of Cell Biology, University of Pittsburgh, PA.
Nearly every cellular function is carried out through a complex network of genetic interactions. These networks and pathways are central to our understanding of biology and are largely learned through genetic screens and protein interaction assays. Yet these methods, as productive as they have been, carry recognized biases and limitations. As a result, the research community is left with fragmented pathways that are missing genes crucial to understanding biology at the molecular level. We have pioneered novel computational methods to identify gene networks using a co-evolutionary signature apparent in gene sequences. This signature, Evolutionary Rate Covariation (ERC), measures correlated rates across a phylogeny of closely related species, allowing us to extract genes with similar evolutionary histories. We calculated a genome-wide ERC dataset over a phylogeny of 18 yeast species. These data contained global evidence that ERC signatures reflect co-functionality between genes, and this signature applies to functionally diverse pathways and complexes throughout the genome. To demonstrate the utility of ERC we inferred functions for 401 uncharacterized yeast genes and confirmed 7 new mitochondrial genes and several modifiers of peroxisome biogenesis. Furthermore, we employed ERC signatures to identify 11 new protein-protein interactions between trafficking adaptor proteins and their cargos at the plasma membrane. Of the 3 interactions tested in our lab to date, each was experimentally confirmed. These functional studies illustrate ERCs potential for gene discovery in any pathway. ERC can also reveal global relationships in the cell through signatures between entire pathways and complexes. To illustrate, we created an ERC-based map for the nuclear pore complex, therein revealing putative functional connections to protein complexes involved in chromatin remodeling, RNA splicing, and the kinetochore. Moreover, these ERC-based predictions were evolutionarily conserved in filamentous fungi and mammals as well. Although these functions are not directly related to nuclear transport, they all indeed have reported connections to the nuclear pore, often using the pore as a site of intracellular organization. Our ERC-based map therefore demonstrates the potential to reveal novel functional connections between nominally distinct pathways. In conclusion, ERC is a unique signature that reflects functional connections between genes and networks and is a powerful complement to classical genetic methods for gene discovery.

333C. The mating pathway as a model for the genetic analysis of complex traits. ***Michael W. Dorrity***[1,2], *Josh T. Cuperus*[2,3], *Christine Queitsch*[2], *Stanley Fields*[2,3]. 1) Department of Biology, University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, University of Washington, Seattle, WA.
Uncovering the genetic underpinnings of complex traits has proven difficult. From crop yield to autism, variants identified in genome-wide association studies (GWAS) explain only a small fraction of the heritable phenotypic variation, leaving a significant gap in our understanding, the so-called missing heritability. Using the mating pathway of Saccharomyces cerevisiae, we seek to develop a model for testing hypotheses about complex trait genetics. We make controlled modifications to the genetic architecture of mating and examine phenotypic output to develop expectations for the translation of genotype to phenotype. The method of deep mutational scanning allows us to measure the effect on mating efficiency due to any of thousands of mutations in genes of the core mating pathway. A given set of mutations can then be tested across several environmental conditions, and in the absence of known genetic buffering mechanisms like the chaperone Hsp90. Mutational scanning of the DNA binding domain of the transcription factor STE12 reveals extensive intra-molecular epistasis, environmentally-responsive mutations, and mutations leading to dependency on Hsp90. We tested over 30,000 STE12 variants for mating efficiency in standard conditions and revealed the baseline distribution of fitness effects along the DNA binding domain, as well as key intra-molecular interactions. Additionally, we subjected the same library to a mating selection with either the Hsp90 inhibitor radicicol or increased temperature (37C). With this approach, we have uncovered and independently validated, in a single gene, several mutations with Hsp90-dependent effects, indicating a role for genetic buffering in maintenance of this complex trait. Mutations buffered by Hsp90 are rare, overlap with temperature-responsive mutations, and tend to be slightly deleterious. Furthermore, multiple buffered mutations are found at a single position, implying a role for protein structure in determining client status. These results point toward a mechanism of establishing Hsp90-dependent interaction.

334A. Functional Evolution of *SIR1* In *Saccharomyces cerevisiae* And Related Budding Yeasts. ***Aisha Ellahi***, *Jasper Rine.* Dept MCB, Univ California, Berkeley, Berkeley, CA.
How exactly do genes evolve different functions throughout the course of their evolution, and how do gene duplication, sequence change, and natural selection influence this process? Using the *SIR1* gene of Saccharomyces cerevisiae and its many divergent paralogs in budding yeast species as a case study, we are investigating the roles that duplication and rapid sequence change play as forces for functional evolution. *SIR1*, along with *SIR2*, *SIR3*, and *SIR4*, functions in the formation

of heterochromatin at the silent mating-type loci *HML* and *HMRa*. *SIR2*, *SIR3*, and *SIR4* also function in silencing at the telomeres, whereas *SIR1* does not. To characterize *SIR1* evolution, we developed genetic tools for *Torulaspora delbrueckii*, a pre whole-genome duplication budding yeast species that contains the most ancestral *SIR1* paralog: *KOS3*. We used mRNA-seq of *sir* mutants in both *S. cerevisiae* and *T. delbrueckii* to uncover loci genome-wide that are subject to *SIR*-based repression. From these data, we found that *Tdkos3* cells exhibited a strong de-repression of *HML*, *HMR*, and telomeric genes, whereas *Scsir1* cells showed a minor phenotype at *HML* and *HMR*, and no de-repression at telomeres. Thus, *TdKOS3* plays a broader role in silencing compared to the derived *S. cerevisiae SIR1*. Additionally, ChIP-seq of Sir proteins in *S. cerevisiae* and *T. delbrueckii* revealed that *Sc*Sir1 is restricted to the silencers of *HML* and *HMR* and absent from telomeres. This is in contrast to *Td*Kos3, whose enrichment overlaps that of *Td*Sir2 and *Td*Sir4, and is also found at several telomeres in the *T. delbrueckii* genome. These data support a model whereby the ancestral *SIR1*, *KOS3*, underwent a dramatic functional shift, going from being essential for silencing to having an establishment-only role in *S. cerevisiae*. Characterizations of *SIR1* paralog function across other experimentally-tractable budding yeast species will illuminate what processes facilitated this functional shift. Future experiments will test hypotheses to reveal specific changes in the *ScSIR1* sequence that led to this dramatic functional shift and to identify the gene that took over *SIR1*s previously essential role in silencing.

335B. Microevolution of a human fungal pathogen in a mouse model of systemic infection. ***Iuliana Ene***[1], *Matthew Hirakawa*[1], *Emily Mallick*[1], *Christina Cuomo*[2], *Richard Bennett*[1]. 1) Molecular Microbiology and Immunology, Brown University, Providence, RI; 2) Broad Institute, Cambridge, MA.
Candida albicans is an opportunistic fungal pathogen and currently the third most common cause of nosocomial bloodstream infections in the US. The ability to adapt to different host niches and evade the immune system is critical for its success as a pathogen. Frequently, this adaptation is mediated by the development of phenotypic variants via genetic changes, a process termed microevolution. While *C. albicans* microevolution has been identified in the development of antifungal drug resistance, this process remains largely unexplored in the context of fitness and virulence in a mammalian host. We tested whether low virulence *C. albicans* strains evolved higher virulence by repeated passaging in a mouse model of systemic infection. We serially passaged three low virulence clinical isolates in a 3-day model of systemic infection for 5 rounds of passaging. While the virulence of two of the passaged strains remained unaltered by serial passaging, the third strain evolved increased virulence. When compared to the initial strain, the evolved isolate caused increased weight loss, decreased motility, and decreased survival in challenged mice. We performed comparative genotyping and phenotyping of the original and evolved isolates to understand the mechanisms responsible for changes in virulence. We found that most of the passaged isolates displayed phenotypic alterations when compared to the original strains, including a trend towards faster growth rates and increased filamentation *in vitro*. Formation of filaments is known to be critical for tissue invasion and the progression of *C. albicans* systemic infection. However, the resistance of passaged isolates to cell wall, oxidative stress, or heat stress remained unchanged. The isolates causing increased virulence displayed enhanced filamentation in response to mammalian host cues such as 37C, high CO_2, or homogenates of organs that are the prime location of disseminated candidiasis, such as the kidney and brain. Our findings indicate that strains with naturally low virulence can display sufficient genetic and phenotypic flexibility to allow for microevolution to occur and that this flexibility leads to increases in the fitness and virulence of *C. albicans* strains. Current analysis is examining genetic differences between parental and passaged strains to define those changes responsible for enhanced virulence. How microevolution events occur in the host and the mechanisms driving phenotypic variation are important for understanding how *C. albicans* can switch its lifestyle from commensalism to pathogenesis.

336C. Phenotypic and Mitochondrial Haplotype Variation in a Local Population of Wild Yeasts. ***Raymond A. Futia***, *Abigail P. Weinberg, Mei-Yi Zheng, Heather L. Fiumera.* Department of Biological Sciences, Binghamton University, Binghamton, NY.
Genetic variation in mitochondrial genomes (mtDNA) can impact cellular and organismal fitness, either independently or through epistatic interactions with nuclear genomes. In Saccharomyces yeasts, previous examination of fitness consequences of mtDNAs has only been between highly divergent strains. The extent that mitochondrial variation contributes to phenotypic differences within local populations of yeasts, where adaptive and coevolutionary processes occur, is unknown. To examine mitochondrial, nuclear, and phenotypic variation in a natural population of yeasts, we used enrichment cultures to isolate Saccharomyces strains from soil and bark samples taken from oak trees of the Binghamton University Nature Preserve in southwestern New York, USA. Ribotyping and sequence analysis of the 5.8S rRNA region was used to identify S. paradoxus strains. MtDNA was purified from each isolate, and digested to reveal restriction fragment length polymorphisms. From 24 independent S. paradoxus cultivars, we observed five unique mtDNA RFLPs, indicating some level of mtDNA sequence variation in this local population of S. paradoxus yeasts. ANOVAs of growth rates obtained in complete synthetic media indicated that strains harboring a common mtDNA variant were phenotypically different from each other, and that mtDNA variation impacts phenotypic variation. We isolated four spontaneous auxotrophic (adenine) mutants from strains with unique mtDNA RFLPs. We are currently examining the fitness impact of

the different mtDNAs when paired with a single S. paradoxus nuclear genotype through karyogamy-deficient matings. In addition, mtDNAs were transferred from these genetically marked strains to a single S. cerevisiae nuclear background to create interspecific mitochondrial-nuclear hybrids. Three unique S. paradoxus mtDNAs supported mitochondrial respiration in S. cerevisiae, nearly to the same level as the S. cerevisiae parent strain. One of the S. paradoxus mtDNAs, however, was maintained by S. cerevisiae, but did not support respiratory growth. Our results demonstrate that extant genetic variation in mtDNAs in local populations of yeasts may affect post-zygotic success of Saccharomyces hybrids and shape evolutionary processes.

337A. Emergence of Novel Ecological Interactions in an Evolving Cooperative Community. ***Robin Green***[1,2], *Chichun Chen*[1], *Jose Pineda*[1], *Wenying Shou*[1]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA.
Cooperation, the costly production of shared benefits, is a pervasive biological phenomenon. For example, host and gut microbiota engage in metabolic exchange; cancer cells may cooperate to promote growth; and microbial pathogens cooperate during infection. Despite its importance, little is known about how phenotypic and genetic diversity changes during the evolution of cooperating systems. We are addressing this question using CoSMO (Cooperation that is Synthetic and Mutually Obligatory), an engineered cooperative system consisting of two reproductively isolated S. cerevisiae strains engaged in nutrient exchange: A lysine-requiring strain that overproduces adenine and an adenine-requiring strain that overproduces lysine. We evolved replicate lines of CoSMO for ~150 generations in a well-mixed minimal medium lacking both adenine and lysine supplements. We predicted that in a well-mixed environment cells would evolve to be more self-serving by adapting to nutrient limitation while simultaneously decreasing benefit production. This is because in a well-mixed environment cooperative benefits are evenly distributed in the population and thus generating more benefits is not necessarily advantageous. To our surprise, we repeatedly found a stable sub-population of methionine auxotrophs that arose from the lysine-requiring cells in multiple independent experiments. Since our minimal media contains no amino acids, these new auxotrophs must acquire their methionine from other cells in the community. We are testing the hypothesis that this new ecological interaction - methionine release and consumption - evolved as a consequence of the environment imposed by the cooperative interactions. Specifically, we are characterizing the release and consumption of methionine in evolved clones, and testing whether this new interaction is adaptive. Taken together, these preliminary data suggest that the cooperative community can rapidly diversify to engage in new and stable ecological interactions.

338B. Tracking the genetic factors of Saccharomyces cerevisiae strains under the selective pressures of the beer brewing process. ***Noah A Hanson***, *Celia Payen*, *Maitreya Dunham.* Genome Sciences, University of Washington, Seattle, WA.
The use of fermentation to create alcoholic beverages is one of mankinds first known biological engineering tasks. Beer, one such product, is made by the saccharification of starch and the fermentation of the resulting sugar to ethanol by yeast. We are exploring the genetic factors that allow yeast to adapt to the selective pressures of this fermentation process, as well as which genes shape the resulting byproduct. To study S. cerevisiaes genomic evolution during fermentation, we are competing thousands of barcoded gene deletion and amplification strains within a medium composed of malted cereal grains (wort). As the beer ferments, samples are collected over time and the frequency of each mutant can be tracked using unique barcodes by high-throughput sequencing. The barcode abundance over time can be used to estimate the fitness impact of each mutation. These data will allow us to identify the genetic components that control growth in wort. We have also begun to explore other phenotypes such as cell morphology and flocculation, and the metabolic by-products that ultimately influence the characteristics of the resulting fermented media (beer). During our pilot project, we explored a number of assays to quantify the glucose and ethanol content of the media, developed a requisite medium, and designed a new fermentation vessel to perform our experiments. This project will expand the potentials of microbial biotechnology, as new targets for genetic engineering have the potential to be identified, and are thus relevant to emerging technological and industrial applications.

339C. Ploidy-regulated variation in biofilm-related phenotypes in natural isolates of *Saccharomyces cerevisiae*. ***Elyse A. Hope***, *Maitreya J. Dunham.* Department of Genome Sciences, University of Washington, Seattle, WA.
The ability of yeast to form biofilms contributes to better survival under stress and affects myriad processes important to human health and industry. In the laboratory, yeast biofilms create significant challenges for many experiments. As a result, biofilm formation has been selected against in most laboratory strains and our knowledge of the genetic basis of yeast biofilm formation remains incomplete. Natural isolates provide a unique testing ground for understanding the spectrum of biofilm-related phenotypes achieved by *Saccharomyces cerevisiae* outside of the laboratory strains. We present a panel of phenotypic assays for 30 haploid and 25 diploid strains in the *Saccharomyces* Genome Resequencing Project natural isolate collection. We show that there is strong qualitative and quantitative variation across five phenotypes: colony morphology, mat formation, flocculation, agar invasion, and polystyrene adhesion. We find that the complexity of many of these phenotypes is only weakly correlated with the others, demonstrating the potential of these assays to evaluate different aspects of biofilm-related morphology. We also show that the diploid phenotypes are

significantly different from their haploid counterparts, in some cases showing increased complexity with respect to the haploid strains contrary to expectations based on the literature about laboratory strains. In addition, we examine gene expression in several known biofilm-related genes and find no strong correlation with any of these phenotypes, also contrary to expectations from literature. These findings clearly demonstrate the utility of examining natural isolates to better understand the correlations between these phenotypes and their relationship to ploidy.

340A. A two loci genetic incompatibility leads to offspring respiratory deficiency within the Saccharomyces cerevisiae species. ***Jing Hou***, *Anne Freidrich, Joseph Schacherer.* Laboratoire de génétique moléculaire, génomique et microbiologie, Université de Strasbourg/CNRS UMR7156, Strasbourg, France.
Understand the molecular basis of how reproductive isolation evolves within a same species offers great insight into the patterns of genetic differentiation as well as the onset of speciation. Previously, we examined the landscape of post-zygotic reproductive isolation within the *Saccharomyces cerevisiae* species by analyzing a large number of crosses among genetically diverse strains, and identified chromosomal rearrangements as the major cause leading to the observed cases of reduced offspring viability. By contrast, genetic incompatibility results from epistatic interactions between diverged genes remained undetected, indicating that this type of mechanism is probably rare and might have a modest effect to the offspring viability under permissive laboratory conditions. To further our understanding of the prevalence of genetic incompatibility relative to the onset of reproductive isolation in this species, we selected 30 crosses that are compatible (offspring viability 90%) on standard laboratory media (YPD), and scored their offspring viability in the presence of different conditions (e.g. carbon sources, temperature, chemicals). One cross between a clinical isolate and the laboratory reference strain S288c was found to be incompatible, showing reduced offspring viability of 75% on media containing non-fermentable carbon sources (e.g. glycerol and ethanol). Using bulk segregant analysis strategy, we mapped the regions and genes that are involved in the incompatibility. We performed allele replacements and confirmed that interactions between incompatible alleles result in respiratory deficiency. This study demonstrates the first example of genetic incompatibility related to specific ecological environment in the *S. cerevisiae* species.

341B. Site-directed changes to *CgPMU1* convert it into a broad range acid phosphatase like *CgPMU2* in *Candida glabrata*. ***Christine L. Iosue***, *Kelly A. Orlando, Sarah G. Leone, Danielle L. Davies, Dennis D. Wykoff.* Biol Dept, Villanova Univ, Villanova, PA.
Inorganic phosphates are required for a wide range of cellular processes, such as DNA/RNA synthesis and intracellular signaling. The *Saccharomyces cerevisiae* phosphate starvation pathway (PHO pathway) is a well-characterized mechanism for regulating phosphate intake and utilization. A marker for PHO pathway activation is the upregulation of the gene encoding an acid phosphatase, *PHO5*. The Pho5 protein is secreted into the periplasmic space, where it cleaves phosphate off of organic phosphate-containing molecules. *Candida glabrata* also exhibits phosphate starvation-inducible phosphatase activity encoded by *CgPMU2*, which appears to have replaced the *PHO5* gene in this species. *CgPMU2* is part of a three-gene family created through gene duplication, but only *CgPMU2* is a phosphate starvation-regulated broad range acid phosphatase.
The *Cg*Pmu proteins are approximately 75% identical. We asked whether we could identify the amino acids that confer broad range phosphatase activity on *Cg*Pmu2. We created fusions of various sections of *CgPMU2* with the ancestral gene, *CgPMU1*, which has no broad range phosphatase activity. This method prompted us to focus on the C-terminal portion of the protein, and use site-directed mutagenesis on various fusions to sequentially convert *Cg*Pmu1 to *Cg*Pmu2. Based on molecular modeling of the Pmu proteins onto a histidine phosphatase crystal structure, two clusters of mutations were found in the lid region of the protein, and may regulate the size and shape of the substrate accessing the active site. The lid region mutations were only able to confer phosphatase activity on *Cg*Pmu1 when combined with one of two point mutations in the tail region of the protein. Interestingly, one of these tail region mutations is a histidine adjacent to the active site of *Cg*Pmu2 that is able to partially replace the conserved active site histidine in *Cg* Pmu2. This result is particularly novel as none of the known histidine kinases that have been examined have this redundant histidine in the active site.

342C. Why do some yeast species have duplicate copies of *GAL*actose network genes? ***Meihua Christina Kuang***[1,2], *Chris Hittinger*[1,2]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, WI.
Gene duplication has long been proposed as a mechanism of regulatory network evolution. Many duplicates appear functionally redundant, and often, we do not fully understand why they are retained. The galactose (*GAL*) utilization network has kept different duplicates in different yeast species after the whole genome duplication (WGD). The *GAL* network in *Sacchaormyce cerevisiae* (*Scer*) has been studied in great details, which allows us to study the functional divergence of this network in related species. *Saccharomyces uvarum* (*Suva*), a relative of *Scer*, has preserved a pair of duplicates that arose from the WGD, *GAL80* and *GAL80B*, whereas the lineage leading to *Scer* has lost *GAL80B*. Why has *Scer* lost *GAL80B*, but *Suva* kept it? Are *SuvaGAL80* and *SuvaGAL80B* functionally distinct? In the *Scer GAL* network,

Gal80 is a repressor that binds to Gal4 to prevent activation of the *GAL* genes in the absence of galactose. Our *Suva* single knock-out data suggests that *SuvaGAL80* and *SuvaGAL80B* are largely redundant over a wide range of galactose concentrations (0.006%~6%) despite only having ~70% amino acid similarity. Preliminary data suggests that they may partially specialize in network induction or maintenance. Surprisingly, the double knock-out (*Suva gal80 gal80b*) does not show rapid growth on galactose as *Scer gal80* does. Instead, faster growth only occurs for less than one cell division, which is then followed by growth arrest for a few hours before growth restarts. This long lag phase (LLP) is concentration-dependent, so we suspect it is due to accumulation of toxic galactose-1-phosphate. However, persistent faster induction appears at low galactose concentrations (0.006%~0.02%) so we hypothesize that *SuvaGAL80* and *SuvaGAL80B* are still both repressors. Allele swaps suggest *SuvaGAL80* and *ScerGAL80* are functionally equivalent in the genetic background of *Suva*, but *SuvaGAL80B* and *ScerGAL80* are not functionally equivalent in the background of *Scer*. In addition to possible functional divergence of *SuvaGAL80B*, changes in other *GAL* genes, such as *SuvaGAL3*, might help explain the retention of *SuvaGAL80B*. Unlike in the lineage to *Scer*, Gal3 in *Suva* has partially or fully maintained kinase activity. Since Gal3 is the co-inducer that relieves the repression of Gal80, we are investigating if it is related to retaining an additional *GAL80* and could have played a role in adaptive conflict resolution.

343A. Single-cell analysis reveals natural variability in a potential bet-hedging trait. ***Colin S. Maxwell***, *Paul Magwene*. Department of Biology, Duke University, Durham, NC.
In uncertain environments, organisms may evolve bet hedging strategies, where individuals randomly develop alternative phenotypes specialized for different modes of the environment. Bet hedging is predicted to evolve when there is high environmental variation, where there are strong trade-offs between the phenotypes, and where these tradeoffs are correlated with fitness in alternate environments. Although the evolutionary conditions that favor bet hedging strategies are well understood, the biological mechanisms underlying such strategies are less well known. We have developed an assay to measure both the division-rate and stress-resistance of individual yeast cells using a flow cytometer. We surveyed more than twenty environmental isolates of *S. cerevisiae* and found extensive differences in both the mean and variance of their division-rate during logarithmic growth in rich media. Furthermore, we found that individuals with a rapid division-rate are more sensitive to mechanical disruption, antibiotics, and reactive oxygen stress than slower-dividing cells. Limited resources available to organisms likely impose a trade-off between growth and stress-resistance, which suggests that bet hedging between quick growth and stress resistance is a likely consequence of evolution in environments with highly variable conditions. We hope to use the tractable genetics of *S. cerevisiae* to unravel the mechanisms underlying this trade-off.

344B. Combining natural sequence variation with high throughput mutational data to reveal protein interaction sites. ***Daniel Melamed***[1,2], *David Young*[2], *Christina Miller*[1,2], *Stanley Fields*[1,2,3]. 1) Howard Hughes Medical Institute; 2) Department of Genome Sciences, University of Washington; 3) Department of Medicine, University of Washington.
Many protein interactions are conserved among organisms despite changes to the sequences that comprise their contact sites, a property that has been used to infer the location of these sites from protein homology. By assessing the functional consequences of substitutions that occur in homologous proteins, inter-species complementation assays can reveal sites of interaction from substitutions that cause deleterious effects. However, the vast majority of the sequence differences within a protein family remain unexplored because of the small-scale nature of these experimental approaches. Here we use high throughput mutational data on the in vivo function of the RRM2 domain of the Saccharomyces cerevisiae poly(A)-binding protein, Pab1, to analyze its sites of interaction. Of 197 single amino acid differences in 52 Pab1 homologues, 17 reduce the function of Pab1 when substituted into the yeast protein. The majority of these deleterious mutations interfere with the binding of the RRM2 domain to eIF4G1 and eIF4G2, isoforms of a translation initiation factor, allowing us to map the interaction sites in Pab1 at single amino acid resolution. Three single amino acid substitutions in yeast Pab1 corresponding to residues from the human orthologue are deleterious and eliminate binding to the yeast eIF4G isoforms. One of these mutations switches the binding specificity of Pab1 from the yeast eIF4G1 to the human eIF4G1. For the other two mutations, we combine epistasis analysis of double mutants and mutual information analysis to predict co-evolving residues, and identify another substitution from the human protein that allows these two mutations to switch binding specificity from the yeast to the human eIF4G1. Finally, we map other deleterious substitutions in Pab1 to inter-domain (RRM2-RRM1) or protein-RNA (RRM2-poly(A)) interaction sites. Thus, the combination of large-scale mutational data and homologous sequence data can be used to characterize interaction sites, even for proteins with limited structural or functional data.

345C. Transport differences in two recently high affinity HXT paralogous. ***A. Mena***[1,2], *E. Barrio*[1], *F. N. Arroyo-López*[3]. 1) University of Valencia Calle Doctor Moliner, 50, 46100 Burjasot, Valencia; 2) IATA-CSIC Institute of Agrochemistry and Food Technology Carrer Catedràtic Agustín Escardino Benlloch, 7, 46980 Paterna, València; 3) CSIC Instituto de la Grasa, Department of Food Biotechnology Av Padre García Tejero, 4, 41012 Sevilla.
Hexose uptake from environment is carried out by a well-described gene family in Saccharomyces cerevisiae known as

HXT. This family consists of 20 genes from HXT1-HXT17, two encoding membrane sensors SNF3 and RGT2 and also a galactose transporter (GAL2). This large 20 encoding permeases family has been derived from one whole genome duplication (WGD) resulted in the ancestor species of some yeast genera including Saccharomyces genus. It has been analyzed that the main contributors of the transport into the cell are genes from HXT1-HXT7 but HXT2. Along Saccharomyces cerevisiae chromosomes we can found a cluster formed by HXT3, HXT6 and HXT7 inside chromosome IV and a second group formed by HXT5, HXT1 and HXT4 inside chromosome VIII. For HXT1 and HXT3 origin it could be hypothesized that they proceed from an ancestral gene as they have similar kinetics and they form a close phylogenetic group; also for HXT4 and HXT6 and HXT7 we could postulate that evolutionary process but for HXT6 an HXT7 an additional duplication process raising two paralogous genes with high degree of homology in nucleotide sequence could succeed recently in evolutionary time; for HXT5 gene, which has an intermediate affinity for glucose, a lost inside chromosome IV could be possible as not so important for cell growing. Concerning to their regulation, they can be classified as low affinity genes when they are expressed under high glucose concentrations and high affinity genes when the expression is related to low glucose levels. Hxt1 and Hxt3 has been classified has low affinity transporters and Hxt6 and Hxt7 Saccharomyces cerevisiae hexose transporters are high affinity transporters and it has been reported that they have more than 90% of degree of nucleotide similarity and form a close phylogenetic group with HXT4. With this information, we have performed phylogenetic analyses to conclude if these genes have been change in their nucleotide or amino acid sequence to infer if their functions also have changed inside Saccharomyces cerevisiae strains from different environments and origins.

346A. Novelty by necessity: Loss of sulfate transport in yeast repeatedly selects for mutations in an uncharacterized transporter *YIL166C* in sulfate-limited environments. ***Aaron W Miller**, Ivan Liachko, Anna B Sunshine, Maitreya J Dunham.* Genome Sciences, University of Washington, Seattle, WA.
The rich variation of earths many species is the product of the development of countless genes with novel function. Despite noveltys importance in evolution it is rarely observed in microbial or metazoan experimental evolution. To explore how proteins with novel function might arise in yeast we preformed a suppressor screen across laboratory evolution timescales in which a sulfate transporter deficient, *sul1sul2* strain was weaned onto incrementally lower sulfate concentrations by serial batch culturing. Since *sul1sul2* cannot grow in sulfate-limited (sul-lim) media, we hypothesized (and found) that evolved mutants capable of growth in sul-lim media have likely generated a novel means to transport sulfate. To search for novel sulfate transporters we first constructed a *sul1sul2* strain and determined that it required 100-fold higher sulfate than a sulfate transport proficient strain. Evolved populations that were eventually capable of growth on sul-lim media were then characterized via whole genome sequencing and also subjected to bulk segregant analysis. Surprisingly all populations (3/3 *S. cerevisiae* and 2/2 *S. bayanus*) contained missense mutations in a previously uncharacterized non-essential transporter, *YIL166C*. *YIL166C* is thought to encode an allantoate transporter with similarity to Dal5p. Interestingly the exact same mutation was observed in both species in a transmembrane region of YIL166C predicted to be involved in substrate transport. Based on these data we hypothesize that *YIL166C* is being converted to an efficient sulfate transporter to compensate for the loss of *SUL1* and *SUL2*. Kinetic and metabolomic analysis is ongoing to determine both the normal function of *YIL166C*, and to characterize the functionality of its evolved derivatives. Collectively this work is an example of how we may be able to use suppressor screens carried out across evolutionary time scales to reveal proteins capable of acquiring new function and thus better understand the potential for novelty in yeast evolution.

347B. The Genetic Architecture of Invasive Growth in a Clinical Isolate of *S. cerevisiae*. ***Helen A. Murphy**[1,2], Debra Murray[2], Jason Smyth[3], Cliff W. Zeyl[3], Paul M. Magwene[2].* 1) Biology, William and Mary, Williamsburg, VA; 2) Biology, Duke University, Durham, NC; 3) Biology, Wake Forest University, NC.
Invasive growth is a form of filamentous growth often found in *Saccharomyces cerevisiae* strains isolated from natural and clinical settings. This phenotype is characterized by elongated cell morphology and substrate invasion, and occurs in response to nutrient scarcity. This complex trait is a model developmental phenotype and is regulated by a number of well-known pathways, including cAMP-PKA, the filamentous growth MAPK pathway, and TOR signaling .We investigated the genetic architecture of this trait in a highly heterozygous clinical isolate, using a two-stage mapping approach based on high-throughput bulk segregant analysis (BSA-seq) in F_2 and F_8 mapping populations. The F_2 analysis suggested the presence of six major loci, and a similar number of minor loci, that contribute to variation in invasive growth. A similar analysis in the F_8 mapping population significantly narrowed QTL regions. Of particular interest was natural allelic variation in TPK2, one of the catalytic subunits of PKA. Similar to other recent studies, these findings suggest that allelic variation that effects cAMP-PKA signaling is a major source of phenotypic variation in *S. cerevisiae*. Comparison of the results from the F_2 and F_8 analyses suggest that at least one locus that was apparent in the F_2 mapping study had been lost due to inadvertent selection on sporulation ability during the generation of the F_8 population, pointing to possible pleiotropic tradeoffs that underlie developmental phenotypes in yeast.

348C. Mitochondrial-Nuclear Epistasis and Coevolution in Natural Isolates of *Saccharomyces cerevisiae*. ***Swati Paliwal***, *Anthony C. Fiumera, Heather L. Fiumera.* Biological Sciences, Binghamton University, State University of New York, Binghamton, NY 13902.
Mitochondrial functions require numerous coordinated interactions between mitochondrial and nuclear genomes. Incompatibilities between divergent mitochondrial and nuclear genomes have been observed within and between species, but the extent that mitochondrial-nuclear (mt-n) epistasis contributes to phenotypic differences in natural populations is unknown. To investigate the contributions of mt-n epistasis to phenotypic variation and coevolutionary processes, we created a novel collection of 100 *S. cerevisiae* yeast strains representing each combination of 10 variable nuclear and mitochondrial genomes from natural isolates. Growth rates were collected in a variety of media conditions, including elevated temperature and fermentable and non-fermentable sugars with or without ROS-generating agents, and analyzed using ANOVA and other biostatistical tests. We found that mt-n epistasis explained a proportion of phenotypic variance in each condition, and that mt-n epistasis had a greater impact than mitochondrial haplotype alone. The patterns of epistasis changed in different environments, demonstrating gene x environment dependence. Naturally occurring mtDNA and nuclear genome combinations provided fitness advantages, including phenotypic plasticity, indicating that coadaptation of mt-n genomes has likely occurred in yeasts. The exchange of mtDNA between strains of similar ecotypes did not lead to decreased epistasis suggesting that selection has favored numerous, potentially constraining, allelic combinations simultaneously within given ecotypes. When naturally occurring mt-n genome combinations were interrupted, mt-n epistasis explained differences in endogenous ROS levels. Exchanging mtDNAs between strains sometimes increased and sometimes decreased endogenous ROS levels, suggesting that multiple molecular mechanisms contribute to phenotypic differences attributed to mt-n epistasis. Our results demonstrate that interactions between mitochondrial and nuclear genomes generate phenotypic diversity in a natural population. This work reveals how previously ignored mitochondrial-nuclear interactions will be important to consider when mapping the genetic basis behind complex traits and can add to our understanding of evolutionary processes.

349A. Investigating reticulate evolution in the *Saccharomyces* genus and repeating it for the bioethanol industry. ***David Peris Navarro***[1,2], *Kayla Sylvester*[1], *Maria Sardi*[1], *William Alexander*[1,2], *Diego Libkind*[3], *Paula Gonçalves*[4], *José Sampaio*[4], *Lucas Parreiras*[2], *Trey Sato*[2], *Chris Hittinger*[1,2]. 1) Department of Genetics, Genome Center of Wisconsin, University of Wisconsin-Madison, WI; 2) Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, WI; 3) Laboratorio de Microbiología Aplicada y Biotechnología, Instituto de Investigaciones en Biodiversidad y Medio-ambiente, INIBIOMA (CONICET-UNComahue), Bariloche, Argentina; 4) Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Cieências e Tecnologia, Universidad Nova de Lisboa, Caparica, Portugal.
In the last decade, the application of new molecular techniques has helped to identify *Saccharomyces* species and suggested many types of reticulated evolutionary events, such as hybridization, interspecies recombination, introgression, horizontal gene transfer, and admixture. Many strains isolated from fermentative environments, such as *S. bayanus*, which was isolated from beer or fermented beverages, and *S. pastorianus*, used in making lager beer, have been misidentified as distinct species when they have chromosome contributions from two or more natural *Saccharomyces* species. These hybridization events likely occurred in the stressful conditions found in industrial environments where hybrids were better suited. These hybrids do not have unique origins, suggesting that hybridization has a clear selective advantage. We have recently described two well-differentiated Patagonian populations of *S. eubayanus*, the wild contributor to *S. pastorianus* (*S. cerevisiae* x *S. eubayanus*) lager-brewing strains. The application of the state-of-the-art phylogenetic methods (Supernetworks and Bayesian concordance factor analysis) illuminated many reticulation events in *S. eubayanus* outside of Patagonia and uncovered the relationship between one population of *S. eubayanus* from Patagonia and its counterpart in *S. bayanus* and *S. pastorianus*. We also recently isolated *S. eubayanus* strains from Sheboygan, WI that originated by intraspecific hybridization (admixture) of the two Patagonian populations, and we used supernetworks to graphically represent the hybridization and recombination in *S. bayanus* and *S. pastorianus* from the brewing industry. With the knowledge gained by understanding the process of hybrid evolution that occurred in winemaking and brewing environments, we are developing new methods to generate artificial hybrids to repeat the hybridization process for the bioethanol industry.

350B. ''Natural and experimental evolutionary dynamics of *Saccharomyces* killer yeast''. ***Magdalena Pieczynska***[1,2], *Ryszard Korona*[2], *J. Arjan G.M. de Visser*[1]. 1) Laboratory of Genetics, Plant Sciences Group, Wageningen, Netherlands; 2) Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, Kraków, Poland.
Viruses are among the most abundant biological entities on earth and found in almost every organism. One of their strategies is to adapt to their host and develop a mutualistic endosymbiosis. In case of the yeast-virus killer system, the cytoplasmic virus produces a compound that is toxic to yeast strains without the virus, while the yeast cell allows for viral survival and reproduction. Transfer of the virus is strictly vertical. So far, little is known about the evolutionary dynamics between yeast host and virus, as well as between killer and non-killer yeast. We are investigating co-evolution in the yeast

killer system at both these levels using laboratory evolution experiments. The study followed the co-evolution of killers and sensitive strains for 500 generations under different conditions: as monoculture and as mixed cultures where either one or both strains were allowed to evolve. We found substantial increases in fitness of all strains in competition with a toxin-resistant reference strain, large changes in virulence of the killer strain and the emergence of resistance in the non-killer strain when both strains were allowed to co-evolve. We investigated the genetic basis responsible for the resistance phenotype, which appeared to be determined by two mutations. By employing cross-infection of viruses between ancestor and evolved killer strains, we were able to track the signature of co-evolution between the virus and its host and identify the cause responsible for alterations in the toxicity.

351C. Natural variation in mutational robustness among strains of *Saccharomyces cerevisiae*. ***Marcin Plech***[1], *J. Arjan G. M. de Visser*[2], *Ryszard Korona*[1]. 1) Institute of Environmental Sciences, Department of Biology, Jagiellonian University, Kraków, Poland; 2) Genetics Lab, Plant Sciences Group, University of Wageningen, The Netherlands.
Background: Organisms are able to remain phenotypically invariant in face of new mutations, even if these are codon-changing mutations and occur in seemingly pivotal regions of their genomes. This ability to evade consequences of deleterious changes is known as genetic or mutational robustness. Among the possible causes of robustness are pervasive redundancy (e.g. via gene duplicates) and high connectivity of genetic circuits. Others ascribe robustness to the presence of genetic capacitors, such as heat shock proteins and chromatin modulators. It has been suggested that the decline of evolutionary fitness associated with acquisition of new mutations may be threshold-like rather than gradual, precisely due to the robustness of their carriers.
Methodology: In this study we have utilized a subset of 20 haploid yeast strains deriving from a previously assembled collection, containing both wild and domesticated isolates. Each strain was treated with EMS (ethyl-methanesulfate), a chemical compound known to randomly introduce transitions of G-C base pairs. We used a gradient of 9 increasing EMS concentrations to simulate varying mutational load. Subsequently we isolated multiple yeast clones per strain, per mating type and treatment, obtaining a collection of 2,240 clones. We measured growth rates in populations treated with mutagen and compared it to that of ancestral lines, such proportion is an estimate of fitness decline and a measure of genetic robustness.
Future plans: The complete genomic sequences are available for all ancestral lines, transcriptomic, proteomic and metabolomic parameters are available for 17 out of 20 strains used in our study. The next step is to correlate available systems parameters with our measures of robustness to reveal the molecular mechanisms of genetic robustness.

352A. Genetic variation acting on protein translation and degradation rates is common in *Saccharomyces cerevisiae*. ***Daniel Pollard***, *Homa Rahnamoun, Scott Rifkin.* Biological Sciences, UCSD, La Jolla, CA.
Yeast cells respond to their environment by coordinating the expression of thousands of genes to produce a specific complement of millions of protein molecules. Natural genetic variation has the potential to impact protein expression dynamics by acting at different levels, including transcription, mRNA stability, translation, and protein stability. The link between genetic variation and mRNA expression divergence is well established, however, the importance of genetic variation acting on protein translation and degradation rates is poorly resolved. To distinguish genetic variation acting directly on mRNA levels from genetic variation acting directly on protein levels, and to dissect how these two types of variation affect different phases of a dynamic cellular response, we measured both mRNA and protein expression dynamics in the laboratory strain S288c and the clinical strain YJM145. We focused on genes in the mating pheromone response network, which is known to harbor heritable variation in gene expression between these strains. We sampled cells from alpha factor treated cultures at time points over the course of twelve hours and then measured mRNA levels using qPCR and protein levels using fluorescence microscopy. A majority of genes examined show differences in protein expression dynamics between strains that can be explained by divergence at the mRNA level. However a minority of genes show divergence in protein expression dynamics that cannot be explained by mRNA divergence, implicating a role for genetic variation acting on translation and protein degradation rates. Our results suggest that genetic variation commonly acts on protein translation and degradation rates, independently of mRNA levels. We will discuss our ongoing efforts to dissect this new class of genetic variants, including distinguishing cis- from trans-acting effects, distinguishing translation from degradation rate effects, mapping causative variants, and scaling up to the whole proteome.

353B. Genome-wide patterns of genetic variation reveal chromosome-scale heterogeneous evolution in a protoploid yeast. *Anne Friedrich*[1], *Paul Jung*[1], *Cyrielle Reisser*[1], *Gilles Fischer*[2,3], ***Joseph Schacherer***[1]. 1) Department of Genetics and Genomics, University of Strasbourg, Strasbourg, France; 2) Sorbonne Universités, UPMC Univ Paris 06, UMR 7238, Biologie Computationnelle et Quantitative, Paris, France; 3) CNRS, UMR7238, Biologie Computationnelle et Quantitative, Paris, France.
Despite the fact that yeast species represent an ideal model system for population genomics, large-scale polymorphism surveys have only focused on two closely related yeast species: *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. Hence little is known about intraspecific diversity and evolutionary history in yeast. To obtain a new insight into genetic

variation within a yeast species, we sequenced the genomes of a large worldwide collection of isolates within a protoploid and distant relative species of *S. cerevisiae*: *Lachancea kluyveri*. We identified 6.5 million SNPs and 243,899 small indels. The genetic diversity is higher whereas the linkage disequilibrium is lower compared to *S. cerevisiae*. Notably, genetic variation in *L. kluyveri* is characterized by chromosome-scale heterogenous evolution spanning an entire chromosomal arm of one megabase. This region, relic of an introgression event, is characterized by a higher genetic diversity, recombination rate as well as a A:TG:C substitution rate, signature of a increased GC-biaised gene conversion. Additionally, our data showed that the overall GC-content of this introgressed region is decreasing whereas the rest of the genome already reached the mutational equilibrium. The chromosomal-scale compositional heterogeneity will persist most probably as the result of an increased GC-biased gene conversion.

354C. Genome sequence and evolution of *Saccharomyces carlsbergensis*, the world's first pure culture lager yeast. *Andrea Walther, Ana Hesselbart,* ***Jurgen W. Wendland***. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Denmark.
Lager yeast beer production was revolutionized by the introduction of pure culture strains. The first established lager yeast strain is known as the bottom fermenting *Saccharomyces carlsbergensis*, which was originally termed *Unterhefe No.1* by Emil Chr. Hansen and used in production in since 1883. *S. carlsbergensis* belongs to group I/Saaz-type lager yeast strains and is better adapted to cold growth conditions than type II/Frohberg-type lager yeasts, e.g. the *Weihenstephan* strain WS34/70. Here, we determined the draft genome sequence of *S. carlsbergensis* based on Illumina sequencing of 8 kb mate-pair libraries. Lager yeasts such as *S. carlsbergensis* are descendants from hybrids formed between a *S. cerevisiae* parent and a parent similar to *S. eubayanus*. Accordingly, the *S. carlsbergensis* 19.5 Mb genome is substantially larger than the 12 Mb *S. cerevisiae* genome. Based on the sequence scaffolds, synteny to the *S. cerevisae* genome, and by using directed PCRs for gap closure we generated a chromosomal map of *S. carlsbergensis* consisting of 29 unique chromosomes. We present evidence for genome and chromosome evolution within *S. carlsbergensis* via chromosome loss and loss of heterozygosity specifically of parts derived from the *S. cerevisiae* parent. Based on our sequence data and via FACS analysis we determined the ploidy of *S. carlsbergensis*. This inferred that this strain is basically triploid with a diploid *S. eubayanus* and haploid *S. cerevisiae* genome content. In contrast the *Weihenstephan* strain is essentially tetraploid composed of two diploid *S. cerevisiae* and *S. eubayanus* genomes. Evolutionary implications for type I and type II lager yeasts are discussed.

355A. Evolutionary Exploration of Yeast Hap4p Regulatory Networks. ***Ruoyu Zhang***, *Xiaoxian Guo, Zhenglong Gu.* Division of Nutritional Science, Cornell University, Ithaca, NY.
Under aerobic conditions, eukaryotes mainly assimilate glucose through the respiration pathway in mitochondria in order to maximize energy yield. However, fermentation is the predominant metabolic pathway for Crabtree-positive yeasts when glucose is present in the environment even oxygen is available. The evolution of this aerobic fermentation is associated with transcriptional reprogramming. The HAP transcriptional regulation complex is involved in the balance between fermentation and respiration in Saccharomyces cerevisiae. Within the HAP complex, the expression of transcription factor Hap4p is induced on non-fermentable carbon sources, suggesting that Hap4p could be the key regulator of aerobic fermentation. Orthologs of Hap4p from different yeasts contain only two short conserved motifs located in the N and C-terminals, while the rest parts of sequences are quite divergent. In this study, we selected the HAP complexes in pre-WGD (Saccharomyces kluyveri and Kluyveromyces lactis) and Post-WGD (Saccharomyces cerevisiae and Saccharomyces bayanus) yeast species, which are responsible for the activation of many genes involved in respiratory metabolism as well as targeting other metabolic functions. In order to elucidate the evolution of yeast Hap4p regulatory networks, we conducted chromatin immunoprecipitation coupling with high-throughput DNA sequencing (chip-seq). Our analysis revealed that Hap4p targets were involved in the respiration pathway in both pre-WGD and post-WGD species. However, Hap4p in pre-WGD species bound to significantly fewer targets than in post-WGD species. In addition, the binding motifs for Hap4p are highly conserved in most species except for K.lactis, which showed massive changes. These results indicated that Hap4p could play a role in shifting respiration to aerobic fermentation in crabtree-positive yeasts. Our study gives new insight into understanding the evolution of transcriptional regulation, and would be a novel contribution to the fields of genetics and cell biology.

356B. The yeast phenome: mapping the functional organization of a eukaryotic cell through an integrative study of genome-wide phenotypic surveys. ***Anastasia Baryshnikova***[1], *Monica Sanchez*[2], *Maitreya Dunham*[2]. 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Department of Genome Sciences, University of Washington, Seattle, WA.
The budding yeast Saccharomyces cerevisiae has been the leading model organism for understanding the functional organization of biological systems. In particular, the yeast deletion collection has empowered hundreds of genome-wide phenotypic screens where mutant alleles of all ~5,000 nonessential genes in the yeast genome have been systematically tested for phenotypes of interest in various environmental conditions. Collectively, these data represent the diversity of the yeast physiological response to genetic perturbation and likely reflect the underlying functional relationships between

genes, pathways and broader biological processes. To investigate these relationships systematically, I compiled a global yeast phenome dataset composed of phenotypic data from all ~200 published systematic surveys of the yeast deletion collection. These data include ~60 phenotypic traits (e.g., growth, morphology, specific metabolite and protein abundance and localization) and thousands of environmental conditions (e.g., chemical compounds, nutrient sources, expression of exogenous proteins, temperature, pH). In addition to published data, this global phenotypic compendium currently includes ~150 unpublished datasets, kindly provided by 42 yeast laboratories. While this resource will be expanded as more data become available, I used the current integrated dataset as part of a preliminary analysis to measure the relative contribution of different phenotypes to predict gene function in Saccharomyces bayanus, a close relative of S. cerevisiae. I anticipate that this analysis will facilitate the functional annotation of the S. bayanus genome and allow us to further explore the evolutionary divergence between related yeast species. Updates on data collection progress, analysis and future data release are available at www.yeastphenome.org.

357C. Multiplexed genome engineering in Saccharomyces cerevisiae. ***Josh Cuperus***[1,2], *Russel Lo*[1,2], *Stanley Fields*[1,2]. 1) Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA; 2) Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA.
Tools for genetic manipulation - such as recombination-mediated mutagenesis and directed evolution - have expanded the usefulness of single-cell organisms for industrial-scale production of biofuels, proteins and other compounds. Using single-cell organisms for industrial production makes the process more environmentally friendly, cost-effective and sustainable. However, additional methods to enhance efficiency are needed to maximize yields or to produce complex compounds. An ideal method would modify an organisms DNA; be high-throughput; allow a measurement of yield without requiring costly measurement techniques; and be readily adaptable to new compounds with high specificity. We are attempting to create a set of promoter elements and transformation procedures in Saccharomyces cerevisiae that enhance the production of proteins and other molecules. Our idea is to create expression elements of differing strength that can be applied in S. cerevisiae to several genes in a pathway. These elements provide a high-throughput method to modify the expression of multiple genes, with the goal of generating some strains that have higher production of a specific compound. To generate these elements, we chose the Tet operator (TetO). TetO sequences are short, and expression can be turned off with tetracycline (or its derivatives). This set of operators allows genes to be activated in yeast by a protein consisting of the Tet repressor fused to the VP16 activation domain. We generated a set of mutant operators that lead to varying levels of expression in yeast. We sought to use lycopene production as a proof-of-principle, because lycopene can be produced in yeast with the addition of three genes from Xanthophyllomyces dendrorhous. We first introduced the three genes into yeast under the control of several versions of TetO and assayed the production of lycopene. Then by generating a library of yeast harboring random TetO variants controlling the three gene, we screened a pool of hundreds of possible promoter combinations for their resultant lycopene production. From these transformations, we found that the most important determinant for lycopene production was high expression from the crtE gene, encoding a geranyl transferase.

358A. WITHDRAWN

359B. Multiple global approaches for deciphering the molecular basis of low temperature adaptation in wine yeast. ***Estéfani García-Ríos***[1], *María López-Malo*[1,2], *José Manuel Guillamón*[1]. 1) Departamento de Biotecnología de los alimentos, Instituto de Agroquímica y Tecnología de los Alimentos (CSIC), Avda. Agustín Escardino, 7, E-46980-Paterna , Valencia, Spainlencia, Valencia, Spain; 2) Biotecnologia Enològica. Departament de Bioquímica i Biotecnologia, Facultat de Enologia, Universitat Rovira i Virgili. Marcel•li Domingo s/n, 43007, Tarragona, Spain.
Low temperature alcoholic fermentations (10-15ºC) are becoming more frequent due to the winemakers tendency to produce wines with more pronounced aromatic profile. However fermentation at low temperature presents some disadvantages: reduced growth rate, long lag phase, sluggish or stuck fermentations. The aim of this study is to phenotype a collection of 27 S. cerevisiae commercial wine strains growing within temperatures (4-45ºC) in both minimal media (SD) and synthetic must (SM) and, taking into account max value, we selected two strains with divergent phenotype in their capacity to grow at low temperature. To confirm this differential phenotype, we design a competition between both strains during wine fermentations. As expected, at low temperature fermentation, the strain showing a good performance out-compete to the strain growing badly in cold, whereas the percentages of both strains were kept around 50% throughout the fermentation process at 28ºC. Finally we aimed to decipher the molecular basis underlying this divergent phenotype by analyzing the genomic, proteomic and transcriptional differences between both strains at low temperature. The up-regulation of genes in the good strain implicated in biosynthesis of sulfur-containing amino acids and S-Adenosilmethionine (SAM) biosynthesis suggest the implication of this pathway in cold adaptation, likely for the requirement of SAM in the biosynthesis of phospholipids (Hickman et al., 2011). We also found genes implicated in glutathione redox reaction, together with the presence of thioredoxins proteins, could be indicating a bigger oxidative stress at low temperature. Moreover, we found glycolysis enzymes of the lower part of the pathway, the part that leads to ATP generation and enhances the glycolytic flux and the fermentative capacity of the strain.

360C. Expression adaptation and gene-dosage response in naturally aneuploid strains of wild *S. cerevisiae*. *James Hose[1], Chris Yong[1], Zhishi Wang[2], Michael A. Newton[2],* ***Audrey P. Gasch***[1]. 1) Laboratory of Genetics & Genome Ctr, U. Wisconsin-Madison, Madison, WI; 2) Departments of Statistics & Biostatistics & Medical Informatics, U. Wisconsin-Madison, Madison, WI.
Gene duplication plays a major role in evolution, by relaxing selective constraints to allow the evolution of gene sequence, function, and regulation. However, duplication of certain genes can pose an initial fitness disadvantage, likely because their amplified expression disrupts expression balance across the transcriptome. Chromosomal aneuploidy represents an extreme case, since chromosome amplification affects many genes simultaneously. Lab strains of yeast are extremely sensitive to chromosomal amplification and are reported to show proportionately higher expression of virtually all amplified genes. However, wild isolates are frequently aneuploid and show little growth disadvantage. We previously showed that expression from most amplified genes is not elevated in wild aneuploid strains - this suggests that natural aneuploid isolates have adapted to aneuploidy, by heritably reducing expression from amplified genes, or that they have an active mechanism to down-regulate expression in proportion to increased gene dosage. To dissect these possibilities, we generated isogenic strain panels for two naturally aneuploid wild isolates and one lab strain, in which diploid strains harbor either two, three, or four copies of affected chromosomes. We performed RNA-seq and DNA-seq analysis across each strain panel and developed a mathematical model to classify genes with heritably lower expression per gene copy versus genes whose expression does not increase with increasing DNA copy number. Thirty to 50% of genes, depending on the strain, showed heritably lower expression per gene copy compared to a close euploid relative. In contrast, up to a third of genes showed disproportionate increases in expression across the wild-strain panels, suggesting that cells actively down-regulated amplified genes in proportion to chromosome copy. One prediction of such a response is that it should occur at genes that are most detrimental when mis-expressed but that it should buffer against gene copy-number variation. We find that genes in this class are under significantly greater evolutionary constraint on expression variation but show elevated rates of copy-number variation in a large panel of wild strains. We propose that aneuploidy tolerance in wild strains facilitates a rapid route to phenotypic variation through chromosomal amplification.

361A. *MKT1* interacts with nitrogen metabolism and mitochondrial signaling pathways to modulate sporulation efficiency variation. ***Saumya Gupta***[1], *Aparna Radhakrishnan[1], Julien Gagneur[2], Himanshu Sinha[1]*. 1) Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400005, India; 2) Gene Center Munich, Department of Biochemistry, Ludwig Maximilians University, 81377 Munich, Germany.
In adverse environmental conditions, yeast initiates meiosis and undergoes sporulation. A previous linkage mapping study identified a coding polymorphism (A89G) in *MKT1*, a gene not known to be associated with sporulation, with 89G allele causative for a significant increase in sporulation efficiency of the S288c strain (Deutschbauer *et al*., 2005). We showed that while Pbp1, a known interactor, was essential for *MKT1* role in SE variation, another interactor *PUF3* contributed to a small fraction of variation only. To identify additional sporulation-specific interactors of *MKT1* (89G), we performed a time-resolved whole-genome expression analysis during sporulation for the two S288c strains differing only for this *MKT1* allele. In a set of sporulation-specific differentially expressed genes between the above two allelic strains, a subset of genes showing early expression changes were identified. The differentially expressed regulators of this early subset were taken as putative molecular intermediates through which *MKT1* (89G) allele could be affecting the sporulation efficiency variation. These regulators included genes involved in various starvation, stress and mitochondrial signaling pathways. Using gene-knockouts and other functional assays, we validated the role of these *MKT1* (89G) allele-specific interactors affecting sporulation efficiency variation. These results identified that nitrogen starvation and mitochondrial signaling pathways genetically interact with *MKT1* (89G) allele to result in better respiration and thereby increasing sporulation efficiency. Current work includes connecting mitochondrial function to nitrogen starvation response in regulating mitochondrial signaling to cause better respiration during sporulation in the presence of *MKT1* (89G) allele. Identification of these causative and correlative effects of *MKT1* allele on the sporulation network provides molecular insights into the functional relationships influencing genetic and phenotypic variation in a quantitative trait.

362B. Identification of novel pathways involved in ploidy maintenance in *Saccharomyces cerevisiae*. ***P.S. Hung***[1,2], *T.L. Sing[1,2], S. Ohnuki[3], B. San-Luis[2,4], J. Paw[2,4], M. Costanzo[2,4], C. Nislow[5], C. Boone[2,4], Y. Ohya[3], G.W. Brown[1,2]*. 1) Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; 2) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; 3) Department of Integrated Biosciences, University of Tokyo, Tokyo, Japan; 4) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 5) Department of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.
In eukaryotes, ploidy maintenance is very important for ensuring genome stability and proper cellular function. Mistakes in spindle checkpoint, spindle pole body duplication and insertion, interaction between kinetochore and microtubules, and cytokinesis failure can lead to an increase in ploidy. Previously, we used flow cytometry to analyze the DNA content of 4646 haploid mutants of *S. cerevisiae* that each contained one non-essential gene deletion. Of these, 177 mutants were

found to be diploid and many of these genes have no known role in ploidy maintenance. We determined that this set of non-essential genes was enriched for the gene ontology (GO) biological processes gene expression (GO: 10467) and chromatin disassembly (GO: 31498). We performed diploidization assays on gene expression mutants and mutants that were morphologically similar to known ploidy maintenance mutants and confirmed that diploidization occurred within 100 generations for 11 of the 13 mutants tested. The majority of diploidized cells were either *MAT***a/a** or *MAT*/, which suggests that these diploids were formed as a result of endoreduplication. The one exception was *msl1*, which was found to undergo a mating-type switching event and became wild-type diploid. Growth rates of 8 deletion mutants were determined over 24 hours and growth advantages were seen in diploidized *rsc2* and *hpr1* strains, but the differences were modest and did not account for the rapid transition from haploid to diploid that was observed. Instead, DAPI staining revealed DNA segregation defects in 4 of the diploidized mutants, which suggests that failed chromosome segregation is the underlying mechanism for the increased ploidy in these mutants. Together, these findings reveal new pathways involved in ploidy maintenance and contribute to defining a complete set of genes responsible for regulation of ploidy.

363C. Cost-effective Genotyping and Karyotyping Using Double Digest-RAD Sequencing in S. cerevisiae, C. albicans and C. glabrata. ***E. Jeffery***[1], *G. Cromie*[1], *A. Forche*[2], *J. Usher*[3], *K. Haynes*[3], *J. Berman*[4], *A. M. Dudley*[1]. 1) Pacific Northwest Diabetes Research Inst, Seattle, WA. USA; 2) Bowdoin College, Department of Biology, Brunswick, ME. USA; 3) University of Exeter, College of Life and Environmental Sciences, Exeter, UK; 4) Tel-Aviv University, Department of Molecular Microbiology and Biotechnology, Tel-Aviv, Israel.
While Next-Generation DNA sequencing platforms have greatly increased the throughput and efficiency of genetic analysis in a wide variety of organisms, large-scale applications of whole genome sequencing remain cost-prohibitive for many labs. Restriction-site Associated DNA marker sequencing (RAD-seq) is a genome reduction strategy that directs DNA sequencing to genomic positions flanking specific restriction enzyme sites. Double-digest RAD-seq (ddRAD-seq) uses two restriction enzymes that are chosen to optimize the number of markers sequenced (2-3% of the yeast genome in our implementation) and to simplify the construction of sequencing libraries. By reducing the proportion of the genome that is sequenced, RAD-seq increases the number of strains that can be multiplexed into a sequencing run, thereby lowering the sequencing cost per strain. Using inline barcodes and indexes, we routinely multiplex ddRAD libraries from 576 S. cerevisiae strains in a single lane of an Illumina Hiseq. At this level of multiplexing, the genomic DNA preparation method is a substantial fraction of the total cost. By reducing the cost of 96-well based yeast genomic DNA preparation, we have been able to ddRAD-seq and analyze several thousand yeast strains in the past year for a cost of approximately $4 per strain. Here, we demonstrate the use of ddRAD-seq for a variety of yeasts (S. cerevisiae, C. albicans, and C. glabrata) and a variety of applications (molecular karyotyping, heterozygosity analysis, and quantitative trait mapping). We also present several data visualization strategies that can facilitate data analysis of a relatively large number of strains with complex molecular karyotypes.

364A. A higher-throughput accurate method for unraveling the genetic basis of Chronological Life Span in yeast. ***Paul P. Jung***[1], *Nils Christian*[1], *Daniel Kay*[1], *Aimée M. Dudley*[2], *Alexander Skupin*[1], *Carole L. Linster*[1]. 1) University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Esch-sur-Alzette, Luxembourg; 2) Pacific Northwest Diabetes Research Institute, Seattle.
Yeast aging is characterized according to two criteria: the Replicative Life Span (RLS) and the Chronological Life Span (CLS). The CLS is defined as the time cells can survive once they have entered senescence. Chronological aging has been investigated in the budding yeast Saccharomyces cerevisiae for decades with a low-throughput approach by determining the Colony Forming Units (CFU) at different time-points in aging cultures. Recently, new high-throughput approaches have emerged using notably outgrowth kinetic assays. Here, we developed a higher-throughput method for CLS characterization using an adapted outgrowth kinetic assay: aging cultures are maintained in four individual 96-deep-well plates (instead of tubes or flasks) and the subsequent growth assay is performed in 384-well plates (instead of 96-well plates). To speed up data analysis and obtain robust results, we developed two freely available softwares: the first one to extract quantitative growth parameters from high-density growth curves, the second one to estimate the CLS by calculating the survival integral, namely the area under the survival curve. Our outgrowth assay produced CLS results that are in good agreement with results obtained by previously published methods and reproduced well-established aging features with, for instance, an increased CLS in a caloric restriction medium. Several aging studies using the reference S. cerevisiae strain and isogenic strains from deletion collections highlighted conserved genes and pathways involved in the control of life span. This reference strain is a phenotypically extreme strain and the use of natural isolates will open new opportunities to investigate the genetics of aging. The application of our higher-throughput CLS assay to a collection of 46 natural S. cerevisiae strains revealed a high variability of life span among those strains. We are currently using an Individual Segregant Analysis approach to determine the genetic basis of chronological aging in yeast by surveying a progeny of more than 500 strains genotyped by RAD-sequencing and isolated from a cross between extremely short- and long-living strains.

365B. Evidence that mutation accumulation does not cause aging in Saccharomyces cerevisiae. ***Alaattin Kaya****, Alexei V. Lobanov, Vadim N. Gladyshev.* Medicine, Harvard University, Boston, MA.
Aging has long been associated with accumulation of damage. While many studies have shown that damage can increase with age, its causal role in the aging process has been difficult to test. Previous studies placed a particular attention on assessing the role of DNA damage in aging, because DNA is the only theoretically non-replaceable molecule in the cell. This is a prolific research area, with many studies demonstrating that DNA damage increases with aging in flies, mice, humans and other organisms. It is also clear that deficiency in certain DNA repair enzymes can shorten lifespan. These studies, however, could not address the causal role of this elevated DNA damage. Indeed, accelerated aging due to DNA repair deficiency does not tell about the role of DNA damage under conditions when DNA repair is fully functional, whereas genetic manipulations that increase lifespan can remodel metabolism, indirectly affecting lifespan. The concept that aging is caused by DNA damage was advanced in the 1950s, but a direct test of this concept has been missing. Here, we subjected Saccharomyces cerevisiae to multiple rounds of replicative aging and assessed de novo mutations in daughters of mothers of different age. Mutations did increase with age, but their low numbers, 1 per lifespan, excluded their causal role in aging. Structural genome changes also had no role. A mutant lacking thiol-peroxidases had the mutation rate well above that of wild-type cells, which did not correspond to the aging pattern. Old wild-type cells lost mitochondrial DNA, whereas mutant cells preserved it, also excluding a causal role of mitochondrial mutations in aging. Thus, DNA mutations do not cause aging in yeast. To our knowledge, this is the first time a causal role of any damage type in aging has been unambiguously tested. We think the same logic should apply to both other damage types and other organisms. If so, the implications of this study are that none of the individual damage types can be a cause of aging, in contrast to what is often discussed in the literature. On the other hand, cumulative damage may still impact the aging process. Thus, our data warrants a radical shift in how the data on manipulations that affect lifespan are interpreted and how we view damage accumulation and causes of aging.

366C. Pooled Segregant Sequencing Reveals Genetic Determinants of Yeast Pseudohyphal Growth. *Qingxuan Song*[1]*, Cole Johnson*[1]*, Thomas Wilson*[2]*,* ***Anuj Kumar***[1]. 1) Dept. of Mol., Cell., and Dev. Biology, University of Michigan, Ann Arbor, MI; 2) Dept. of Pathology, University of Michigan Medical School, Ann Arbor, MI.
The pseudohyphal growth response is a dramatic morphological transition and presumed foraging mechanism wherein yeast cells form invasive and surface-spread multicellular filaments. Pseudohyphal growth has been studied extensively as a model of conserved signaling pathways controlling stress responses, cell morphogenesis, and fungal virulence in pathogenic fungi. The genetic contribution to pseudohyphal growth is extensive, with at least 500 genes required for filamentation; as such, pseudohyphal growth is a complex trait, and linkage analysis is a classical means to dissect the genetic basis of a complex phenotype. Here, we implemented linkage analysis by crossing each of two filamentous strains of Saccharomyces cerevisiae (1278b and SK1) with an S288C-derived non-filamentous strain. We then assayed meiotic progeny for filamentation and mapped allelic linkage in pooled segregants by whole-genome sequencing. This analysis identified linkage in a cohort of genes, including the negative regulator SFL1, which we find contains a premature stop codon in the invasive SK1 background. The S288C allele of the polarity gene PEA2, encoding Leu409 rather than Met, is linked with non-invasion. In 1278b, the pea2-M409L mutation results in decreased invasive filamentation and elongation, diminished activity of a Kss1p MAPK pathway reporter, decreased unipolar budding, and diminished binding of the polarisome protein Spa2p. Variation between SK1 and S288C in the mitochondrial inner membrane protein Mdm32p at residues 182 and 262 impacts invasive growth and mitochondrial network structure. Collectively, this work identifies new determinants of pseudohyphal growth, while highlighting the coevolution of protein complexes and organelle structures within a given genome in specifying complex phenotypes.

367A. Genetic Incompatibilities in Hybrid Yeast. ***Samuel M. Lancaster****, Miatreya J. Dunham.* Genome Sciences, University of Washington, Seattle, WA.
Hybrid organisms can yield important insights into the forces underlying evolution and have significant societal impact. The budding yeast in the sensu stricto clade of Saccharomyces readily hybridize across species. To investigate genetic incompatibilities in hybrid yeast, we hybridized the *Saccharomyces cerevisiae* deletion collection to WT *Saccharomyces bayanus* var *uvarum* to scan the hybrid genome for hybrid-specific haploinsufficiency and for haploproficient *S. bayanus* alleles that confer a fitness benefit onto the hybrid organism. This collection was then competed *en masse* in chemostats under three different nutrient limiting conditions. To determine the effect of hybridization on fitness, we created several thousand isogenic *S. cerevisiae* x *S. bayanus* hybrids, each only differing with a unique DNA barcode. Fitnesses were determined in each competition by using an Illumina platform to sequence the barcode of each strain over several generations. Using the fitnesses of the WT hybrid collection as the null distribution, we determined many of the hybrid strains with deleted *S. cerevisiae* alleles did indeed have significant fitness increases or decreases. Furthermore, several of these hybrid deletion strains demonstrate antagonistic pleiotropy between nutrient limitations. Our findings also show that hybrid yeast contain multiple recessive alleles that if uncovered confer fitness benefits. Follow up experiments have demonstrated that these effects are background specific and reciprocal hemizygosity analysis has shown that they are allele

specific as well. The mechanisms for the beneficial recessive mutations are still under investigation, but appear to include better tolerances to proteotoxic stress. Together, these findings elucidate the fitness effects of hybridization in *Saccharomyces*, they show the near complement of genetic incompatibilities in hybrid yeast, and suggest selection mechanisms for hybrid speciation.

368B. An Evaluation of High-Throughput Approaches to QTL Mapping in Saccharomyces cerevisiae. *Stefan Wilkening[1], **Gen Lin**[1], Emilie Fritsch[1], Manu Tekkedil[1], Simon Anders[1], Rauel Kuehn[2], Michelle Nguyen[2], Raeka Aiyar[1], Michael Proctor[2], Nikita Sakhaneko[3], David Galas[3,4], Julien Gagneur[3], Adam Deutschbauer[5], Lars Steinmetz[1]*. 1) European Molecular Biology Laboratory, Genome Biology Unit, 69117 Heidelberg, Germany; 2) Stanford Genome Technology Center, Palo Alto, California 94304; 3) Pacific Northwest Diabetes Research Institute, Seattle, Washington 98122; 4) Luxembourg Centre for Systems Biomedicine, University of Luxembourg, L-4362, Esch-sur-Alzette, Luxembourg; 5) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720.
Dissecting the molecular basis of quantitative traits is a persistent challenge in genetics and is essential for understanding complex diseases. Because of several confounding factors that render the dissection of complex traits in humans difficult, yeast has been used as a powerful model system. Still, even in yeast and other model organisms, precisely determining causative genes and their interactions has remained elusive, due in part to difficulty in narrowing intervals to single genes and in detecting epistasis or linked quantitative trait loci. These difficulties are exacerbated by limitations in experimental design, such as low numbers of analyzed individuals or of polymorphisms between parental genomes. We address these challenges by applying three independent high-throughput approaches for QTL mapping to map the genetic variants underlying 11 phenotypes in two genetically distant Saccharomyces cerevisiae strains, namely (1) individual analysis of 700 meiotic segregants, (2) bulk segregant analysis, and (3) reciprocal hemizygosity scanning, a new genome-wide method that we developed. We reveal differences in the performance of each approach and, by combining them, identify eight polymorphic genes that affect eight different phenotypes: colony shape, flocculation, growth on two nonfermentable carbon sources, and resistance to two drugs, salt, and high temperature. Our results demonstrate the power of individual segregant analysis to dissect QTL and address the underestimated contribution of interactions between variants and also reveal confounding factors like mutations and aneuploidy in pooled approaches. This is the first comprehensive study of different QTL mapping methods and it provides a roadmap for future dissection of quantitative traits.

369C. An Assessment of Terminal Phenotypes in *S. cerevisiae* Using Synthetic Genetic Array and High-Content Screening. ***Dara Lo**[1], Jason Moffat[1,2], Brenda Andrews[1,2], Charles Boone[1,2]*. 1) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada.
Our group has developed and applied the Synthetic Genetic Array (SGA) method to map networks of synthetic lethal interactions using a colony size-based read-out. Although an easily assayable phenotype, colony size does not provide information about the mechanistic underpinnings of the slow growth or death phenotypes caused by genetic interactions. To explore the range of cellular phenotypes associated with severe genetic lesions, such as synthetic lethality and perturbation of essential gene function, we have developed methods that integrate SGA analysis with high-content screening (HCS) to enable the high-throughput, quantitative assessment of changes in subcellular morphology that culminate in defects in cell growth or death. First, we are developing our analysis pipeline by using SGA-HCS to examine the terminal phenotypes associated with mutation of essential genes. This strategy involves using SGA to introduce fluorescent markers of key cellular compartments into strains carrying temperature sensitive alleles of essential genes, and performing live cell imaging using high-throughput confocal microscope over a time course of 24 hours. As a pilot study, we have assessed 27 mutants of essential genes using 12 GFP reporters of subcellular structures and compartments. Preliminary results indicate that terminal phenotypes are temporally dynamic and reflect both early changes in subcellular morphology that tend to be specific the mutated gene, and later defects more generally associated with cell death. Second, we are using methods we have developed for high throughput isolation of temperature sensitive alleles to isolate ts alleles of non-essential genes in different synthetic lethal backgrounds. These assays will provide us with functional information that will allow us to determine the mechanisms behind terminal phenotypes, to map novel connections between biological processes, and to gain further insight into the functional wiring of the cell.

370A. Beyond Beer and Wine: Isolating Wild Yeast from Unroasted Cacao and Coffee Beans. ***Catherine Ludlow**[1], Cecilia Garmendia Torres[2], Amy Sirr[1], Michelle Hays[3], Colby Field[4], Gareth Cromie[1], Eric W. Jeffery[1], Aimée M. Dudley[1,3]*. 1) Pacific Northwest Diabetes Research Institute, Seattle, Washington, USA; 2) Institut de génétique et de biologie moléculaire et cellulaire, Strasbourg, France; 3) Molecular and Cellular Biology Program, University of Washington, Seattle, WA USA; 4) Montana State University, Bozeman, Montana, USA.
S. cerevisiae has long been used as a model system to address a wide variety of biological questions, but research has been largely limited to a small set of closely related laboratory strains, prompting some to seek out wild yeast populations. Even so, most wild *S. cerevisiae* strains currently available to researchers have been isolated in Europe and North America, with

little representation from Asia, Oceania, Africa and South America. We sought to overcome this problem by isolating *S. cerevisiae* from commonly imported food products associated with fermentation. Here, we demonstrate the feasibility of isolating large numbers of wild *S. cerevisiae* strains from unroasted cacao and coffee beans. Using this approach, we have considerably expanded the number of strains from under-sampled areas. Our RAD-seq analysis of 200 strains from coffee and cacao suggests that these populations are admixed, the result of matings between European and Asian strains. Also, unlike population structures of strains isolated from vineyards and North American oak trees, the coffee and cacao strains have population structures that strongly reflect the geographic area from which they were isolated.

371B. Exploring the endocytic pathway by combining high-throughput genetics and high-content microscopy. ***Mojca Mattiazzi Usaj***[1], *Matej Usaj*[1], *Marinka Zitnik*[2], *Blaz Zupan*[2], *Brenda Andrews*[1], *Charles Boone*[1]. 1) The Donnelly Centre, University of Toronto, Toronto, Ontario, Canada; 2) Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia.
Endocytosis is a highly conserved fundamental cellular process that controls the lipid and protein composition of the plasma membrane, and the exchange of the majority of molecules between a cell and its environment. It is a complex process that depends on an intricate network of interacting proteins and precise coordination of molecular events, and serves as a link between many intracellular signalling networks. We have combined synthetic genetic array (SGA) analysis with high-throughput confocal fluorescence microscopy and quantitative image analysis to assess the phenotypes of cortical actin patches, endosomes and vacuoles for yeast *S. cerevisiae* deletion mutants and temperature sensitive (TS) essential gene mutants, covering approximately 5400 open reading frames (~90% of the yeast genome). Our systematic and automated approach confirmed previously known components of the endocytic pathway and identified new genes, including some previously uncharacterized genes, involved in the process of endocytosis. Here, the work pipeline, results and ongoing efforts will be presented.

372C. Navigating the chemical space of large compound libraries using high-throughput chemical genomics. ***Jeff Piotrowski***[1], *Sheena Li*[2], *Raamensh Deshpande*[3], *Scott Simpkins*[3], *Justin Nelson*[3], *Jacqueline Barber*[2], *Hiroyuki Osada*[2], *Minoru Yoshida*[2], *Chad Myers*[3], *Charlie Boone*[4]. 1) Great Lakes Bioenergy Research Centre University of Wisconsin - Madison, USA 53726; 2) Center for Sustainable Resource Science, RIKEN, 2-1 Hirosawa, Wako, Saitama, Japan 351-0198; 3) Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN 55455, USA; 4) 4.Banting and Best Department of Medical Research and Department of Molecular Genetics, Donnelly Centre, University of Toronto, 160 College St., Toronto, ON, Canada M5S 3E1.
The gulf between described bioactive compounds and those with defined molecular targets continues to grow through combinatorial chemistry and compound isolation. While target-centric strategies are widely adopted for high-throughput drug discovery, the successes of these initiatives have been modest. An unbiased, cell based assay that may provide functional information across the landscape of cellular targets. Saccharomyces cerevisiae has become a powerful model for chemical genomics by virtue of the wealth of genomic tools available for this organism. To allow large-scale exploration of compound collections via chemical genomics we created a high-throughput system that incorporates a novel drug-sensitive and miniaturized deletion collection, highly multiplexed barcode sequencing (768-plex), and a set of customized computational tools. Using this system, we have screened over 14,000 compounds, including 10,000 from a unique natural product library, the RIKEN NPDepo. Leveraging the predictive power of the yeast genetic interaction network, we have functionally annotated over 800 high-confidence bioactive compounds. Linking them to their target cellular processes and generated a functional map of the RIKEN NPDepo. The functional landscape of this collection reveals a diversity of targeted process across the compounds, with unexpected enrichment for compounds targeting mitosis and glycosylation. Using the structural data combined with the chemical genomic profile, we have a means of determining structure activity relationships between compounds and derivatives. With this system we can screen thousands of compounds inexpensively and rapidly. High-throughput chemical genomics allows an unbiased whole-cell approach to drug library screening that can link active compounds to their cellular targets.

373A. Transcriptional and post-transcriptional analyses of the iron deficiency response in *Saccharomyces cerevisiae*. ***Antonia Maria Romero***[1], *Daniel A Medina*[2], *Jose García-Martinez*[3], *M.Teresa Martínez-Pastor*[2], *Jose E. Pérez-Ortín*[2], *Sergi Puig*[1]. 1) 1Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (IATA) Consejo Superior de Investigaciones Científicas (CSIC), Paterna, Valencia, E-46100, Spain; 2) Department of Biochemistry and Molecular Biology. Universitat de València. Ave. Doctor Moliner, 50; E-46100 Burjassot (Valencia) Spain; 3) Department of Genetics. Universitat de València. Ave. Doctor Moliner, 50; E-46100 Burjassot (Valencia) Spain.
Iron is an essential element for most organism because participates as an essential cofactor in numerous cellular processes such as respiration, DNA synthesis and repair, ribosome biogenesis and other metabolic pathways. Previous studies have shown that both transcriptional and post-transcriptional mechanisms regulate the response of the budding yeast *Saccharomyces cerevisiae* to iron deficiency. The Aft1/Aft2 transcription factors activate the expression of a group of genes referred as the iron regulon involved in high-affinity Fe uptake at the plasma membrane, Fe mobilization from

vacuolar stores, and metabolic reprogramming of Fe-consuming pathways. Two mRNA-binding proteins denoted Cth1 and Cth2 specifically bind to AREs within the 3UTR. To ascertain the relative contribution of transcriptional and post-transcriptional strategies to the adaptation to iron scarcity, we have determined in parallel the mRNA levels (RA) and transcription rates (TR) for the entire yeast transcriptome during the progress of iron deprivation. To obtain TR genome-wide we have used the Genomic Run-On (GRO) technique, whereas mRNA decay rates (DR) have been calculated from the RA and TR experimental data. Our results confirm previous observations indicating that the iron regulon is up-regulated in response to iron deficiency due to an increase in TR, whereas genes that participate in the mitochondrial electron transport chain are down-regulated by an increase in their DR. Furthermore, we observe how genes implicated in translation decreased their TR during the progress of iron scarcity. We are currently performing further analyses to decipher how the different regulatory mechanisms coordinate to optimize the yeast response to iron deficiency.

374B. Transcriptional variations among Saccharomyces cerevisiae strains harboring different alleles of a transcription factor Yrr1. ***Xiaoqing Rong-Mullins***[1], *Wei Zheng*[2], *Hogune Im*[2], *Erin Mitsunaga*[2], *Michael Snyder*[2], *Jennifer Gallgher*[1]. 1) Biology, West Virginia University, Morgantown, WV; 2) Genetics, Stanford University, Stanford, CA.
Transcription factors regulate transcription of genes and thus cellular responses to exogenous chemicals. Yrr1 is a transcription factor that regulates responses to 4-nitroquinoline 1-oxide (4NQO) and to glycerol as the sole carbon source in Saccharomyces cerevisiae. Different Yrr1 alleles confer variable responses to 4NQO and glycerol in different strains such as S96 (related to S288c) and YJM789 (a genetically divergent clinical isolate). S96 allele of Yrr1 confers sensitivity to 4NQO and faster growth utilizing glycerol as the sole carbon source. In contrast, the Yrr1 allele from strain YJM789 confers resistance to 4NQO and slower growth on glycerol. In this study, global transcriptomic changes in response to 4NQO and glycerol in strain S96 and YJM789 were measured by RNA-Seq. In S96, 136 loci were differentially expressed (q-value 0.01) in response to 4NQO, 8 of which were novel loci that were not previously identified as genes; 1654 loci were differentially expressed in response to glycerol, 49 of which were novel. In YJM789, 138 loci were differentially expressed in response to 4NQO, 3 of which were novel; 1762 loci were differentially expressed in response to glycerol, 56 of which were novel. ChIP-Seq of Yrr1 protein showed that 523 enriched regions were within 1000 bp upstream and downstream of those differentially expressed loci in one or both strains in response to 4NQO and/or glycerol. For example, one region enriched in both strains under all the conditions was located upstream of *VID24* (on reverse strand) and *PHO88* (on forward strand), both of which encode peripheral proteins involved in transmembrane transport. Vid24 is located at vacuole import and degradation vesicles and regulates fructose-1,6-bisphosphatase targeting to the vacuole; it was upregulated in response to 4NQO in S96 and to glycerol in YJM789. Pho88 is involved in phosphate transport and was downregulated in response to glycerol in both S96 and YJM789. The promoter region of *SNQ2*, previously known to confer sensitivity to 4NQO, was enriched in Yrr1 ChIP-Seq, which was consistent with a previous finding that *SNQ2* is regulated by Yrr1. *SNQ2* did not show significantly differential expression in response to 4NQO in either strain, but was significantly upregulated in response to glycerol in YJM789. The combined data of RNA-Seq and Yrr1 ChIP-Seq provided candidate loci likely to be regulated by Yrr1 in response to 4NQO and/or glycerol.

375C. Comparative analysis of stress responses in diverse wild yeast strains. ***Nikolay S Rovinskiy***[1,2], *Dana J Wohlbach*[1,2], *Jeff Lewis*[1,2], *Maria I Sardi*[1,2], *Wendy S Schackwitz*[3], *Joel A Martin*[3], *Shweta Deshpande*[3], *Chris Daum*[3], *Trey K Sato*[2], *Audrey P Gasch*[1,2]. 1) Genetics, University of Wisconsin-Madison, Madison, WI; 2) Great Lakes Bioenergy Research Center; Madison, Wisconsin 53706; 3) US Department of Energy Joint Genome Institute; Walnut Creek, California 94598.
Different strains of wild yeast S. cerevisiae have variable tolerance to the different types of stresses. Understanding differences in stress tolerance can shed light on defense mechanisms. In this work we studied the transcriptome responses to stress in one lab and three wild type strains. We subjected these strains to heat and ethanol stress with consequent RNA-seq to measure the difference in expression across strains and stresses. We applied a general linear model to identify differentially expressed genes. Model-based clustering of differential expressed genes allowed us to point out the physiological processes that may be responsible for difference in stress response of wild type strains. Using de novo transcriptome assembly we have shown that all four strains contain non-S288c sequences and the strain with the highest resistance to stress has the largest amounts of these transcripts. Most of these transcripts are mapped to sequences which are similar to the yeast pseudogenes which may be expressed in studied strains and contribute to their stress tolerance.

376A. Chromatin organization in quiescent yeast. ***Mark T. Rutledge***[1,2], *Jon-Matthew Belton*[3], *Mariano Russo*[2], *Job Dekker*[3], *James R. Broach*[2]. 1) Dept. of Molecular Biology, Princeton University, Princeton, NJ; 2) Dept. of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA; 3) Program in Systems Biology, Dept. of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA.
Most cells exist primarily in a quiescent state generally defined by cessation of division with maintenance of viability. Saccharomyces cerevisiae cells starved for different essential nutrients enter distinct quiescent states depending on the

nutrient for which they are starved as well as the method of inducing the starvation. These growth state changes are accompanied by host of large-scale differences in various molecular phenotypes such as transcript, protein, and metabolite levels. To further understand the physical context of these gene expression changes within the nucleus we assayed chromatin changes associated with the transition from exponential growth to glucose-starved quiescence. At the nucleosome level (assayed by MNase-Seq) we see that with increasing length of starvation comes 1) an increase in median nucleosome density at promoters and genes relative to the other parts of the genome, 2) a decrease in the global precision of positioning of nucleosome peaks, and 3) more global variability in the distances between nucleosome peaks. These chromatin changes seen in quiescence generally occur in two phases corresponding to a quick-response phase that happens within thirty minutes after starvation and a long-term adaptation phase that occurs by one day of starvation. Furthermore, promoter nucleosome density changes show a negative correlation with transcript level differences between the two states, and highly repressed genes show promoter nucleosome gains not seen in genes at other levels of expression. At the whole chromosome level (assayed by Hi-C) upon switching from growth to quiescence we see a dramatic decrease in centromere-centromere interaction frequency and a large increase in interaction frequency of subtelomeric-subtelomeric regions both within and between chromosomes. Interestingly, short range intra-chromosomal interactions (below roughly 50 kb) show large decreases in frequency during quiescence while long range intra-chromosomal interactions (above roughly 50 kb) show a large increase in frequency. Overall, most inter-chromosomal interactions only show a small increase in interaction frequency. We conclude that the DNA's physical arrangement within the nucleus undergoes large-scale reorganization at lower- and higher-order scales upon the switch to quiescence.

377B. Genomic approaches to understanding Saccharomyces cerevisiae tolerance to lignocellulosic-hydrolysate toxins. ***Maria I. Sardi***, *Dana J. Wohlbach, Audrey P Gasch.* University of Wisconsin-Madison, Madison, WI.
The increased demand for alternative fuels is driving the development of more efficient and economical production of biofuels from plant biomass. One of the challenges with this source of energy is that the chemically treated plant material, known as lignocellulosic hydrolysate, contains a variety of toxic compounds known to affect fermenting microbes by decreasing growth, metabolism, and alcohol production, making lignocellulosic fermentation an inefficient process. In this study, I screened a panel of 90-sequenced wild and domesticated Saccharomyces cerevisiae strains coming from diverse geographic and ecological backgrounds, for growth in ammonium fiber expansion pretreated corn stover hydrolysate (ACSH) in both aerobic and anaerobic conditions. We found that strains ranked differently when growing with or without oxygen, revealing that the lack of oxygen has a significant impact on lignotoxin tolerance. Surprisingly we found that wild yeast strains out-performed industrial strains evolved to ferment a variety of alcoholic beverages and that are known to thrive in anaerobic conditions. This finding underscores the importance of studying wild yeast strains in order to understand how cells deal with a multi-stress environment. Moreover, our screen revealed a quantitative phenotype highly variable among strains, indicating that a genetic component lies behind the differences in growth and tolerance to this media. Using available and generated genome sequences for these strains, we developed a pipeline for genome wide association (GWA) analysis. Ongoing RNA-seq analysis will be used to understand the mechanism of stress tolerance and to inform the results of GWAS. Together, this work will fill a missing area of scientific knowledge regarding mechanisms of surviving lignotoxins, which is relevant to our understanding of natural environments, evolution, and engineering of efficient strains to ferment plant biomass.

378C. A phenomic assessment of sub-cellular morphology in *S. cerevisiae* using Synthetic Genetic Array analysis and high-content screening. ***Erin B. Styles***[1], *Lee Zamparo*[1], *Karen Founk*[1], *Oren Kraus*[1,2], *Dogus Altintas*[3], *Marco Graf*[4], *Daniele Novarino*[5], *Tina Sing*[1], *Grant W. Brown*[1], *Marco Muzi-Falconi*[5], *Brian Luke*[4], *David Shore*[3], *Brendan Frey*[2], *Zhaolei Zhang*[1], *Charles Boone*[1], *Brenda J. Andrews*[1]. 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada; 2) Electrical and Computer Engineering, University of Toronto, Toronto, ON, Canada; 3) Department of Molecular Biology, NCCR Program "Frontiers in Genetics", and Institute of Genetics and Genomics in Geneva, 30 quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland; 4) DKFZ-ZMBH Alliance, University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany; 5) Dipartimento di Bioscienze Universita' degli Studi di Milano, Milano, Italy.
High-throughput (HTP) multi-channel fluorescence microscopy enables analysis of subcellular changes in the proteome of living cells in response to genetic & environmental perturbations. The budding yeast *S. cerevisiae* is a premier model for the application of genome-wide approaches due to ongoing development of tools & reagents for systematic functional genomics & proteomics. These collections, coupled with methods for automated yeast genetics such as Synthetic Genetic Array (SGA) analysis, mean that genetic interactions can be rapidly assessed using a variety of phenotypic readouts. We have undertaken a systematic assessment of sub-cellular morphology using a comprehensive set of fluorescent markers of cellular compartments, & sensitized backgrounds. We have coupled SGA with automated microscopy to quantitatively assess the abundance and localization of proteins in response to thousands of genetic perturbations. Our strategy involves using SGA to introduce fluorescent markers of key cellular compartments, along with sensitizing mutations, into the yeast deletion collection & temperature sensitive mutant collections, & performing live cell imaging on the mutant arrays using

HTP microscopy. As proof-of-principle, we assessed DNA damage repair pathways by evaluating Rad52-GFP foci in single mutants & a number of chemically or genetically sensitized backgrounds (phleomycin treatment, *sgs1*, *yku80*). Analysis identified 179 mutants with high levels of Rad52 foci, & analysis of sensitized backgrounds yielded an additional 167 mutants. As an example of this, a *vid22 sgs1* mutant population has elevated levels of Rad52 foci relative to either single mutant. Further analysis revealed that *vid22* confers sensitivity to DNA damaging agents & elevated DNA:RNA hybrids. This emphasizes the utility of sensitized genetic backgrounds. We are now expanding our analysis to include 20 subcellular compartments, with the goal of producing a global view of subcellular morphology.

379A. Genetic basis for Saccharomyces cerevisiae biofilm in liquid medium. *Kaj S. Andersen[1], **Laura G. R. Sørensen**[1], Rasmus Bojsen[2,3], Martin W. Nielsen[2,3], Michael Lisby[1], Anders Folkesson[2,3], Birgitte Regenberg[1].* 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Department of Systems Biology, Technical University of Denmark, Copenhagen, Denmark; 3) National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark.
Biofilm forming microorganisms switch between two forms: free-living planktonic and sessile multicellular. Sessile communities of yeast biofilms in liquid medium provide a primitive example of multicellularity and are clinically important because biofilm tend to have other growth characteristics than free living cells. We investigated the genetic basis for yeast, Saccharomyces cerevisiae, biofilm on solid surface in liquid medium by screening a comprehensive deletion mutant collection in the 1278b background and found 71 genes that were essential for biofilm development. Quantitative northern blots further revealed that AIM1, ASG1, AVT1, DRN1, ELP4,FLO8, FMP10, HMT1, KAR5, MIT1, MRPL32, MSS11, NCP1, NPR1, PEP5, PEX25, RIM8, RIM101, RGT1, SNF8, SPC2, STB6,STP22, TEC1, VID24, VPS20, VTC3, YBL029W, YBL029C-A, YFL054C, YGR161W-C, YIL014C-A, YIR024C, YKL151C, YNL200C, YOR034C-A, YOR223W regulated biofilm through FLO11 induction. Almost all deletion mutants that were unable to form biofilms in liquid medium also lost the ability to form surface-spreading biofilm colonies (mats) on agar though the conditions for the two phenotypes are very different and 69% also lost the ability to grow invasively. The protein kinase A isoform Tpk3p functioned specifically in biofilm and mat formation. In a tpk3 mutant, transcription of FLO11 was induced three-fold compared to wild type, but biofilm development was absent, suggesting that Tpk3p regulates FLO11 positive post-transcriptionally and negative transcriptionally. The study provides a resource of biofilm-influencing genes for additional research on biofilm development and suggests that the regulation of FLO11 is more complex than previously anticipated.

380B. An all-in-one yeast library - creating a new toolbox for studying the proteome. *Uri Weill, **Ido Yofe**, Maya Schuldiner.* Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.
The use of fluorescent reporter proteins enables tracking and quantifying proteins within single cells, and permits detailed measurements of the protein and organelle characteristics. In *Saccharomyces cerevisiae*, epitope tags can be inserted by genetic alterations either internally or at the N or C termini of proteins using simple PCR-based recombination techniques, therefore making this yeast an ideal model organism for systematic investigations of the proteome at the single cell level. Protein C' tagging is the most widely used since it allows to maintain the protein natural promoter. Nevertheless, proteins that have low expression levels or that contain C maturation or processing signals will not be visualized correctly. This shortcoming secludes many proteins from high-throughput screening, which would facilitate their characterization (about one third of the yeast proteome has not been visualized to date). Thus, in order to characterize proteins never seen before using C'-tagging, we are constructing, for the first time, a library of N' tagged yeast genes, focusing on ~1400 proteins of interest as a first step. Our library has two dramatic advantages: First, we use a seamless tagging approach that enables us to investigate proteins under the regulation of both a generic promoter and their native promoter, as it allows the removal of the tag selection cassette. Therefore, on the one hand we are able to visualize low abundant proteins for the first time, and on the other hand also investigate proteins under their endogenous regulation. Second and most importantly, our N tagging strategy establish our library as an acceptor library- a basis for countless other libraries. To date, the construction of each yeast collection was done using an extremely laborious and expensive procedure that discourages researchers from perusing many biological questions. Using a simple cross of our library with a strain harboring a donor tag, we are able to swap the N tag with any other tag of interest, be it another fluorescent protein, an inducible promoter, an affinity tag and so forth. Our N' tag acceptor library constitutes a powerful new tool, creating in essence an endless pool of possible libraries, and will make way for many experimental avenues for systems level investigations of the proteome.

381C. Establishing the yeast as a model for human disease. ***Neta Agmon**, Leslie Mitchell, Jef Boeke.* Bioch & Mol Pharm, Institute of Systems Genentics, NYU Langone Medical Center, New York, NY.
Decades of research have led to the development of a large array of high throughput libraries and technologies for screening in yeast. In combination with the most resent advances in synthetic biology, yeast cells can be manipulated to serve as either a factory for producing a desired product or as a tool to study cellular pathways. We evaluated whether we could express an entire human metabolic pathway in yeast. We chose the purine biosynthesis pathway as our first working model. This pathway is highly conserved from yeast to humans. In humans there are 20 disorders associated with the

pathway and the associated metabolic network, and include a variety of symptoms, including gout, autism, blindness, loss of immunity, kidney failure and different neurological abnormalities. Importantly, most of these diseases lack any established treatment. We propose to use yeast cells for expressing human mutant alleles of human diseases in order to study their effect on their associated metabolic network as well as on other pathways in the cell and for screening for possible treatments. Using a synthetic biology approach, we are swapping the entire purine biosynthesis network of the yeast with the cognate human genes. We have already created a yeast expression vector/neochromosome, which includes half of the human genes in the network, namely the full de novo adenine biosynthesis pathway, under the transcriptional control of their cognate yeast promoters and terminators. 100 percent of these complement for their yeast orthologs deletion phenotypes. Additionally, we are progressively deleting all the corresponding yeast genes in this pathway. We will thus ultimately attempt to swap all 26 genes in the entire network, producing a yeast cell with a humanized purine metabolic network. This will enable future investigation of the entire network in a variety of yeast based high-throughput methods as well as screening for new drugs for the pathway associated diseases. The Purine metabolic network can serve as a proof of principle for our ability to take any human disease and its associated pathway/gene network, whether it exists naturally in the yeast or not, and establish a yeast model for the disease.

382A. Mapping resistance to targeted cancer therapeutics using a deep mutational scanning approach in Saccharomyces cerevisiae. ***Ethan Ahler***, *Douglas Fowler*. Department of Genome Sciences, University of Washington, Seattle, WA.
Targeted drugs that strike at the specific drivers of cancer have revolutionized treatment. Unfortunately, clinical responses are often short-lived, with resistance developing in a majority of patients. Resistance commonly occurs as a result of point mutations in the target oncogene that prevent drug-target interactions. Although some resistance mutations for targeted therapies are well characterized, most remain unidentified, particularly for new and emerging drugs. A complete understanding of how a proteins sequence influences sensitivity to drugs could help rationally guide therapeutic decisions and improve next-generation drug design. To assess the resistance of all possible single mutants in a protein of interest to a drug, we are applying deep mutational scanning. Deep mutational scanning leverages selection for protein function and high-throughput DNA sequencing to measure the activity of hundreds of thousands of variants of a protein simultaneously. Initially, we will apply this strategy to the promising drugs dasatinib, bosutinib and saracatinib, which target Src kinase. To select for Src inhibition, we have developed a system in S. cerevisiae where expression of active Src variants results in unchecked phosphorylation and substantially reduces yeast growth. This Src-mediated growth defect is rescued in a dose-dependent manner by Src inhibitors. We will generate a library of the 10,720 singly mutated Src variants and transform this library into yeast. Src expression followed by library outgrowth in the presence of each inhibitor will cause yeast harboring sensitive variants to grow quickly due to relief of Src-mediated growth inhibition, whereas yeast harboring resistant variants will grow slowly. We will use high-throughput DNA sequencing to determine the frequency of each variant at multiple time points of the outgrowth and based on these frequencies, we will calculate a functional score for each variant. We will repeat this experiment at multiple concentrations of each drug and use the resulting functional scores to generate EC50 values for every variant. Because the yeast cellular environment differs from human, we will verify the resistance phenotypes by investigating the activity of a subset of variants that span the range of resistance in a human cancer cell line. Thus, we will generate a complete sequence-resistance map for Src, which will describe a resistance and activity score for each amino acid variant at every position. We intend to use this map to help direct patient-specific therapeutic decisions and contribute to the development of next-generation inhibitors that can bypass current mechanisms of resistance.

383B. Synthetic dosage lethality of *CTF4* and the identification of orthologs amplified in breast cancer. ***Eric Bryant***[1], *John Dittmar*[2], *Robert J. D. Reid*[2], *Rodney Rothstein*[2]. 1) Biological Sciences, Columbia University, New York, NY 10027; 2) Genetics and Development, Columbia University Medical Center, New York, NY 10032.
An underappreciated therapeutic target in cancer is copy number amplification (CNA). In fact, most frequently amplified genes are disregarded passenger alterations, because they do not drive the survival and transformation of the cancer, and are therefore not directly targetable with a drug. However, CNA can cause vulnerabilities that are targetable via synthetic dosage lethal (SDL) genetic interactions, regardless of driver/passenger status. An SDL interaction occurs when overexpression of a gene causes synergistic cell death when paired with a normally viable mutant. Thus, any CNA that results in overexpression can potentially be targeted by drug inhibition of an SDL partner. While identifying SDL interactions in human cells remains challenging, this task is easily performed in *Saccharomyces cerevisiae*. Here we present an analysis that uses CNA, gene expression, mutation frequency, and cross-species complementation data, to prioritize SDL screens in yeast. This analysis has identified 120 orthologs commonly amplified and overexpressed in breast cancer, including orthologs present in an amplified region associated with reduced patient survival. Additionally, a screen for SDL interactions of *CTF4* (whose human ortholog, *WDHD1*, is found to be upregulated in aggressive breast cancers) found *CTF4* overexpression to cause sensitivity to loss of genes involved in replication fork progression, DNA repair, and DNA damage checkpoint signalling. These SDL interactions align with the known role of *CTF4* in tethering DNA polymerase to the DNA replication fork. Further experiments found *CTF4* overexpression to cause an increase in

Rad52-YFP foci occurance (an indicator of DNA repair centers), and an increase in a-faker frequency (an indicator of genomic instability). Interestingly, *CTF4* overexpression and *ctf4* share many synthetic genetic interactions, and both show a genomic instability phenotype. However, *CTF4* overexpression appears to be unique in causing sensitivity to loss of 911 DNA damage checkpoint signalling. In sum, *CTF4* is a "Goldilocks" gene whose expression must be "just right" to maintain genomic stability.

384C. Identification of biological roles for yeast Rbd2, a member of a subfamily of rhomboid proteins implicated in human disease. ***Christa L. Cortesio***[1], *Eric B. Lewellyn*[1], *Nathaniel I. Krefman*[1], *Catherine C. Wong*[2], *John R. Yates 3rd*[2], *David G. Drubin*[1]. 1) Molecular and Cell Biology, University of California-Berkeley, Berkeley, CA; 2) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.
Rhomboid proteins are a family of multi-pass, integral membrane proteins found in all domains of life. The rhomboid family consists of both active proteases and catalytically inactive proteins. Members of this family regulate important cellular signaling events and biological processes. A rhomboid sub-family, which includes human RHBDD1 and RHBDD2, has been implicated in several types of advanced cancers; yet, the functions of these proteins during normal physiology and disease progression are not known. We report that the yeast member of this rhomboid subfamily, Rbd2, regulates actin assembly during clathrin-mediated endocytosis (CME) through a PtdIns(4,5)P2-dependent mechanism. Combined genetic and biochemical studies demonstrated that Rbd2s cytosolic tail binds directly to PtdIns(4,5)P2 and is sufficient for Rbd2s role in actin regulation. Rbd2 functions in a pathway with the PtdIns(4,5)P2-generating lipid kinase Mss4 and the PtdIns(4,5)P2 binding, syndapin-like, F-BAR protein Bzz1 to regulate myosin recruitment and actin polymerization at endocytic sites. Due to similarity in sequence, similar predicted topology and cellular distribution, we have identified RHBDD2 as the likely human homologue of Rbd2 and show that the human RHBDD2 cytosolic tail can regulate the onset of actin polymerization during CME in yeast. This work identifies *in vivo* functions for Rbd2 in three, highly conserved and interconnected processes relevant to human health and disease: lipid organization, cytoskeletal function and endocytic regulation.

385A. Elucidating the metabolic basis of cancer using yeast as a surrogate system: A model for tumorigenesis. ***Jaswandi U. Dandekar***[1], *Abhay Kumar*[2], *P. Jayadeva Bhat*[1]. 1) Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India; 2) Thyrocare Technologies Limited, Navi Mumbai.
Use of model systems for genetic studies has helped to gain a better insight into human gene functions. Yeast is one of the most commonly used model systems for such studies due to its ease of handling, genetic manipulations and shorter generation time. Yeast has been used widely as a surrogate system to study various human diseases like mitochondrial myopathies, peroxisomal disorders, neurodegenerative diseases and cancers. A shift from glucose oxidation to fermentation, even in the presence of oxygen, is a universal feature of cancer cells, known as the Warburg effect. p53, the tumor suppressor protein in humans, has been identified as one of the key regulators of this metabolic shift. Similar to the cancer cells, fermentation is the default pathway of glucose metabolism in *Saccharomyces cerevisiae*. Hence, we surmised that *S. cerevisiae* can be tailored to understand the mechanism of this metabolic shift that occurs in cancer cells. For this purpose, we overexpressed human p53 from *GAL* promoter in *S. cerevisiae*. We observe that overexpression of p53 causes apoptosis of yeast cells in a carbon source dependent manner i.e. p53 causes apoptosis only in cells that utilize non-fermentable carbon source, such as glycerol but not in cells that utilize fermentable carbon source such as galactose or sucrose. We have also expressed the human p53 from galactose inducible promoter in *Kluyveromyces lactis*, an aerobic yeast. For the first time, we show that p53 overexpression in *K. lactis* leads to growth inhibition, independent of the carbon source. This observation gives an insight into the fact that p53 might be interfering with mitochondrial function, as unlike *S. cerevisiae*, *K. lactis* cannot bypass the mitochondrial pathway of metabolism and hence experiences growth inhibition upon p53 overexpression. Disruption of *AIF1*, a known human orthologue involved in yeast apoptosis, abrogated the p53 dependent apoptosis in *S. cerevisiae*. Using the above model, currently we are investigating whether fermentative mode of metabolism has any role to play in the escape of apoptosis in cancer cells.

386B. Interactions of *HEM25* with Genes Encoding for the Mitochondrial Transporters Family (*SLC25*). ***J. Noelia Dufay***[1], *J. Pedro Fernández-Murray*[1], *Christopher R. McMaster*[1,2]. 1) Biochemistry & Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada; 2) Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.
Congenital sideroblastic anemia is a rare hematological disease primarily caused by mutations in genes that result in a defect in heme/hemoglobin biosynthesis. The most recently identified mutated gene is *SLC25A38*. We determine that *SLC25A38* encodes a mitochondrial glycine transporter. Glycine is imported into the mitochondria where it is used in the first enzymatic step in heme synthesis. We went on to identify new genetic interactions with *SLC25A38* to further our understanding of which cellular processes can affect cell fitness when *SLC25A38* is mutated. The yeast *Saccharomyces cerevisiae* was used as a model to identify genetic interactions, as the heme biosynthetic pathway is highly conserved from yeast to humans and genetic interactions can be quickly and accurately identified using this model. The homologue of *SLC25A38* in *S. cerevisiae* is *HEM25*. Strains were constructed using standard genetic crosses to create 32 double mutants

where the *HEM25* gene and the gene coding for every mitochondrial member of the *SLC25* family was inactivated. We found that a small subset of *SLC25* members have a negative genetic interaction with *HEM25*. These mutations were in genes that provide molecules for use by the electron transport chain, and the negative genetic interaction phenotype of these double mutants was revealed primarily under respiratory conditions, a growth condition that requires ATP to be generated exclusively by the mitochondrial electron transport chain. The results of this work may help discover new pathways and processes that affect cell fitness due to inherited heme biosynthesis deficiency in humans.

387C. Yeast - it simply has a lot to say about human disease. ***Selina S. Dwight***, *Kalpana Karra, J. Michael Cherry, SGD Project.* Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305.
Science has long approached a complex system by exploring a simpler system that exhibits similar functionality. What happens when the complex system itself becomes more penetrable? Human gene products have been known for decades to have counterparts in *S. cerevisiae*, and detailed knowledge about these *S. cerevisiae* genes has provided clues to basic cellular functions in humans and other higher organisms. In the past decade, however, technological advances in genomic sequencing and other methods have allowed more direct studies in human cells. Contrary to what might be expected, the result is not that budding yeast is less consequential as a model organism, but that increasing information about human genes verifies and expands the connections between the two organisms, allowing human research to more readily leverage yeast knowledge. For instance, identification of regions in human genes with similarity to yeast prion-like domains led to the discovery of a mutation in an evolutionarily conserved residue in a gene that co-segregates with ALS. Conversely, increasing knowledge about human biological processes also suggests areas for further study in yeast. As an example, mutation of mitochondrial DNA associated with aging, disease, and anticancer agents has prompted studies of pathways in yeast that sense the nutritional state of the cell and affect mitochondrial function. Lastly, while yeast may be a relatively simple system, yeast research has witnessed its own advances in technology that serve to further its relevance as a model organism - such as chemogenomic profiling of systematic deletion strains to identify potential drug targets and the mechanisms of action by which compounds exert their effects on disease. As researchers continue to elucidate functional homology between yeast and humans, and to employ yeast as a tool for discovery, it becomes increasingly important to highlight and connect this information. The *Saccharomyces* Genome Database (SGD, www.yeastgenome.org) is building a new class of information called Species Connections towards this goal through the incorporation of OMIM (Online Mendelian Inheritance in Man) homologs and disorders, phenologs, drug interactions, and functional homology studies. As a first step, we have entered human orthologs of yeast genes and their corresponding OMIM disease associations into YeastMine, SGD's powerful search tool. YeastMine makes it easy to explore connections between yeast genes that share the same phenotypes and their human disease orthologs, to find the human orthologs and disease relationships for a known set of interactors for a yeast gene, and to search for a disease or human gene name and return any counterparts in yeast.

388A. Deep mutational scanning of amyloid □ to illuminate mechanisms o
interactions. ***Vanessa E. Gray***, *Jason J. Stephany, Douglas M. Fowler*. Genome Sciences, School of Medicine at the University of Washington, Seattle, WA, USA.
Amyloid () aggregation plays a key role in Alzheimers disease; chaperone proteins can inhibit A aggregation and influence the course of the disease. Despite the importance of A aggregation in disease etiology, our understanding of the determinants of aggregation is sparse and largely derived from in vitro studies. To overcome these gaps in knowledge, we will use deep mutational scanning to measure the aggregation propensities of tens of thousands of A variants in the presence and absence of various chaperones in vivo. A library of A variants will be subject to selection for aggregation using an assay that links yeast growth to A aggregation. Aggregation-prone variants will decrease in frequency, whereas non-aggregating variants will increase in frequency. We will use high-throughput DNA sequencing to track the frequency of each A variant during the selection, enabling us to assign an aggregation propensity score to each variant in the library. Our study will provide the first large-scale, in vivo mutational dataset of A, illuminating the physiochemical properties of amino acids that negate, promote or do not effect A aggregation. We will assess the concordance of our data with available models of aggregate structure and aggregation mechanism to determine which models are accurate. Next, we will study how chaperones influence A aggregation by measuring each variants aggregation propensity in the presence of excess chaperones. Initially, we will focus on the impact of human alpha B crystallin (BC) and yeast heat shock protein 70 (Ssa1) on A aggregation by overexpressing them in our yeast assay. The result will be a large-scale chaperone rescue data set, enabling us to identify A variants whose aggregation is altered by presence of each chaperone protein. By analyzing the physicochemical properties of these chaperone-sensitive variants, we will characterize the molecular attributes that facilitate chaperone-A interactions. Comparisons of these attributes will reveal the features of A sequence that dictate chaperone engagement. Thus, we will clarify the in vivo physicochemical determinants of A aggregation and interactions between A and chaperones.

389B. Solving protein structure with large-scale mutagenesis. *Katherine A Sitko, Douglas M Fowler,* ***Margaret L Griset***. Genome Sciences, School of Medicine at the University of Washington, Seattle, WA.

Because a proteins three-dimensional structure defines its function, improved methods for resolving structure are an important objective in molecular biology. For example, the structures of many pharmaceutically relevant proteins are difficult to characterize with current experimental approaches. Computational techniques that predict structures from amino acid sequences obviate problematic physical manipulation of proteins, but are unreliable. Computational prediction improves, however, when supplemented with limited structural data inferred from a proteins evolutionary history: when amino acids at two distant positions within a sequence have coevolved across many species, these positions are usually spatially adjacent in the proteins three-dimensional structure. This method is limited by the scarcity of related proteins among species. We propose using large-scale mutagenesis to generate protein functional data by introducing mutations into an essential *S. cerevisiae* gene to create an exhaustive library of variants of a protein, each of which contains one or two random mutations in its amino acid sequence. We will then apply deep mutational scanning, a method we developed that measures the functional consequences of hundreds of thousands of variants simultaneously. In a deep mutational scan, selective pressure for protein function is applied to a library of variants while high-throughput DNA sequencing tracks the frequency of each variant. Deleterious variants decrease in frequency whereas beneficial variants increase in frequency; these changes in variant frequency are used to calculate functional scores. We have shown that the functional scores of two singly mutated variants usually predict the functional score of the doubly mutated variant containing both single mutations. Double mutants with unexpectedly high or low scores denote an interaction between the mutated positions, and we hypothesize that such interacting positions are close in space. Spatial constraints revealed by large-scale mutagenesis could augment computational approaches to produce accurate models. The proposed method would be especially useful for determining otherwise intractable three-dimensional protein structures.

390C. Systematic identification of human/yeast cross-species complementation pairs. ***Akil Hamza***, *Phil Hieter*. Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada.
While the pace of discovery of somatic mutations in tumor genomes has rapidly accelerated, deciphering the functional impact of these variants has become rate-limiting. Using cross-species complementation, we propose that model organisms like the budding yeast, *Saccharomyces cerevisiae*, can fill this gap and serve as a platform for studying human genetic variants. In this instance, human/yeast cross-species complementation refers to the ability of a human gene to complement its yeast orthologue and rescue a loss-of-function phenotype. Yeast provides an attractive model system for a complementation assay given that this single-celled eukaryote shares most of its essential cell biology with humans, has a well-characterized genome and is amenable to genetic manipulation and genomic engineering. As such, we outline here an experimental approach to identify a comprehensive list of human genes which when expressed in yeast can complement a loss-of-function phenotype. Given that rescue-of-lethality is the easiest phenotype to assay, we have focused initially on yeast essential genes. Briefly, by utilizing gateway cloning, human cDNAs in gateway-compatible entry clones are systemically shuttled to a yeast expression vector. In turn, complementation of yeast essential genes is assessed by examining rescue-of-lethality of the yeast knockout following sporulation of the haploid-convertible heterozygous diploids. The result is a list of candidate human genes whose somatic mutations can be characterized directly in yeast while also providing a list of complementing genes as a resource to the field.

391A. Anticancer ruthenium complex KP1019 induces the heat shock response in yeast. *Laura Stultz*[1], *Alexandra Hunsucker*[2], *Evan Grovenstein*[2], *James Mobley*[3], ***Pamela Hanson***[2]. 1) Chemistry Department, Birmingham-Southern College, Birmingham, AL; 2) Biology Department, Birmingham-Southern College, Birmingham, AL; 3) Mass Spectrometry/Proteomics Facility, University of Alabama at Birmingham, Birmingham, AL.
According to early clinical trials, the anticancer ruthenium complex indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate (III) - also known as KP1019 - is a promising alternative to platinum-based chemotherapeutics, as it stabilized disease progression in 5 of 6 evaluable patients without any dose-limiting toxicity. Although KP1019 has previously been shown to damage DNA in colorectal cancer cells and in yeast, the drugs ability to bind proteins in biophysical assays and in cultured mammalian cells suggests additional modes of action. To elucidate the drugs mechanism(s), we examined the cellular response to KP1019 by using mass spectrometry to compare protein expression in yeast treated with 0 or 80g/ml KP1019. Of the 3676 different proteins detected across three biological replicates, 178 proteins exhibited statistically significant (p0.05) increases in expression and a mean induction of at least 2-fold. Consistent with previous proteomic studies on the DNA damaging agent MMS, KP1019 treated yeast significantly induced genes involved in deoxyribonucleotide metabolism, ribosome biogenesis, and stress responses. Flow cytometry of GFP-tagged strains verified induction of several heat shock proteins (hsps) that are targets of the heat shock transcription factor Hsf1. Moreover, KP1019s ability to induce expression of a heat shock element (HSE) *lacZ* reporter construct, suggests that the drug activates Hsf1. When compared to untreated controls, yeast pre-treated with KP1019 displayed higher rates of survival following heat shock at 52C, suggesting that KP1019-dependent induction of hsps is physiologically significant. Future experiments will explore the mechanism of Hsf1 activation as well as the contribution of hsps to tolerance of KP1019, potentially leading to therapeutic approaches involving co-administration of KP1019 and hsp inhibitors.

392B. Development of a fission yeast-based high throughput screen to target heterologously-expressed cAMP signaling pathway proteins. *Ana Santos de Medeiros, Alexander Magee, Grace Kwak, Sam Rivera, Jordan Vanderhooft, Rachel Gottlieb,* ***Charles S. Hoffman***. Biology Dept, Boston College, Chestnut Hill, MA.
We describe the construction and characterization of a PKA-repressed *fbp1*-GFP reporter that can be used to characterize the activity of heterologously-expressed cAMP pathway proteins and to screen for small molecules that reduce PKA activity in the fission yeast *Schizosaccharomyces pombe*. We show that expression of mammalian adenylyl cyclases (ACs) in *S. pombe* activates PKA to repress the GFP signal and that co-expression of the human GNAS1 leads to a stimulation of AC activity. In addition, the activity of cyclic nucleotide phosphodiesterases (PDEs) increases the GFP signal. As such, high throughput screens can be carried out using strains that express multiple components of a mammalian cAMP pathway in order to detect inhibitors of either an AC or GNAS1, as well as activators of a PDE. There is significant need for such compounds as chemical probes to dissect various cAMP-regulated processes as mammals express 10 different ACs and 16 different PDEs that have activity against cAMP. We will discuss results from a pilot screen of 10,000 compounds carried out in a 384 well micro titer dish format for activators of the human PDE4D3 enzyme that is involved in inflammation and cognition.

393C. S. cerevisiae as a Platform for High Throughput Screening and Variant Analysis of the Sphingosine-1-Phosphate Receptor Family. ***Jacob Hornick***[1]*, Son Nguyen*[4]*, Pam Benegal*[3]*, Shen-Shu Sung*[2]*, James Broach*[1]. 1) Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA; 2) Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA; 3) Department of Molecular Biology, Princeton University, Princeton, NJ; 4) Union College, Schenectady, NY.
We have used yeast as a platform for exploring two aspects of the human sphingosine-1-phosphate (S1P) family of G protein-coupled receptor receptors (S1PR): (1) targeting its role in cancer progression (2) characterizing the pharmacologic consequences of natural variants of S1PR genes in the human population. The S1PR family is critical for many cellular processes including: cytoskeletal rearrangement, cellular motility and invasion, angiogenesis, immunological processes, and vascular maturation. Moreover, progression of large granular lymphocyte (LGL) leukemia depends on S1PR subtype 5 overexpression. Since knockdown of S1PR5 reverses the transformed phenotype of LGL leukemic cells but other S1PR subtypes are essential, we sought antagonists that are specific solely for the S1PR5 subtype. We constructed yeast strains, each of which functionally expressed one of the five S1PR receptor subtype, and used them to screen for S1PR5 specific antagonists. A preliminary screen of the Spectrum Collection (MicroSource Discovery Systems) yielded 42 candidate S1PR5 specific antagonists, and five were chosen for further study. Molecular modeling of the compound set has provided support for the preliminary screen and identified other potential candidates. In a second study, we have examined the effect of natural variants in S1PR genes on the signaling properties of the receptors. SNPs within the receptor family, rs61734752 in S1PR1 and rs3745268 and rs117064827 in S1PR2 had significant effects on the potency of the natural ligand S1P. Interestingly, these variants were not near the ligand binding domain of the receptor. Variant rs35483143, which occurs within the C-terminus of S1PR5, eliminates the ability of the receptor to couple to G12, one of the two cognate G through which the receptor signals. We have confirmed in mammalian cells the signaling defect of this variant. We are currently addressing the phenotypic consequences of these variants through studies in model vertebrates and through analysis of existing human genetic data.

394A. Ribosomal Perturbation as a Strategy for Improving Protein Biogenesis in Cystic Fibrosis. ***Mert Icyuz***[1]*, Kathryn E Oliver*[2]*, Eric J Sorscher*[2]*, John L Hartman IV*[1]. 1) Genetics, University of Alabama at Birmingham, BIRMINGHAM, AL; 2) Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham,BIRMINGHAM, AL.
Cystic fibrosis is an autosomal recessive disease caused by loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR), an ATP-binding cassette (ABC) transporter with epithelial chloride channel function. Deletion of Phenylalanine-508 (F508) in CFTR results in protein biogenesis defects evidenced by improper folding, aggregation, misprocessing and aberrant localization with increased degradation. This single mutation affects 90% of cystic fibrosis (CF) patients, and thus strategies to improve its biogenesis constitute potential treatments for the disease. We recently reported development of a yeast phenomic model of the CFTR-F508 gene interaction network utilizing the homologous F670 mutation in the yeast ABC transporter, YOR1 (Yeast Oligomycin Resistance). As shown by Elizabeth Millers laboratory, the Yor1-F670 protein exhibits a similar biogenesis defect to that seen for CFRT-F508. The yor1-F670 allele was introduced to the S. cerevisiae gene deletion library by SGA and analyzed by quantitative high throughput cell array phenotyping (Q-HTCP) to identify genetic modifiers, using oligomycin sensitivity as a readout of Yor1-F biogenesis. Hits from the screen were homologous to F508 protein regulators previously characterized in the CF literature, demonstrating evolutionary conservation within gene interaction networks that modulate biogenesis of ABC transporters harboring the F mutation. More recent experiments have shown that rpl12a-0 (or deletion of other ribosomal proteins) affects oligomycin sensitivity only in the context of yor1-FRT allele, further confirming its interaction with yor1-F670. Based on ribosomal protein observations in the yeast phenomic model, the effect of RPL12 knockdown in CF bronchial epithelial cells was examined, and rescue of CFTR-F508 biogenesis was robustly observed to an extent greater than the

leading drugs in development for treatment of CFTR-F508-related cystic fibrosis. Polysome analysis in yeast revealed that ribosome assembly and translation efficiency were not grossly affected by Rpl12 deletion. Ongoing studies are to assess ribosome occupancy of yor1-FRT mRNA by qRT-PCR to infer the effect of the Rpl12a deletion on yor1-F670 translation rate and to use time-lapse fluorescence microscopy to directly visualize the effect of Rpl12 deletion on biogenesis of a conditionally expressed yor1-FRT-GFP allele.

395B. Arg-Trp-Arg based peptidomimetics with antifungal activity. ***Camilla E Larsen***[1], *Camilla J Larsen*[2], *Henrik Franzyk*[2], *Birgitte Regenberg*[1]. 1) Department of Biology, University of Copenhagen , Copenhagen , Denmark; 2) Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.
Abstract: Due to increased occurrence of yeast infections and spoilage in the food industry, there is an unmet need for new antifungals. The arginine--(2,5,7-tri-tert-butylindol-3-yl)alanine -arginine (R-Tbt-R) motif was previously found useful in the design of an antifungal tripeptide. Here, an array of peptidomimetics based on this motif was investigated for antifungal and hemolytic activity. The five most promising modified tetrapeptides contain an additional C-terminal hydrophobic residue, and these were found to exhibit antifungal activity against *Saccharomyces cerevisiae* and the spoilage yeast *Zygosaccharomyces bailii*. Determination of their killing kinetics revealed that two of the compounds were efficient fungicides. Testing against cells from an *S. cerevisiae* deletion mutant library allowed identification of resistant mutants. These experiments indicated that interaction with the fungal-specific sphingolipids, mannosylinositol phosphorylceramide MIPC and mannosyl di-(inositol phosphoryl) ceramide M(IP)2C, most likely is a crucial step in the mode of action of four of the peptidomimetics. Another analogue containing two Tbt residues appears to disrupt both yeast and human membranes, however, it was the only compound also displaying antifungal activity towards pathogenic *Candida* spp. Interestingly, the lack of activity of the four other compounds towards *Candida* spp. was shown to be due to degradation or sequestering by the yeast. These four compounds all had low hemolytic activity, and due to their ultrashort nature they may have potential as preservatives.

396C. Expression, modification and characterization of fungal natural products in *Saccharomyces cerevisiae*. *C. Harvey, **J. Li**, M. Hillenmeyer, R. Davis.* Stanford Genome Technology Center, Stanford University, Stanford, CA.
The enormous diversity of natural products in thousands of species, including many fungi, present great opportunities for exploring natural products synthesis, discovering compounds of medical interests, and optimizing the production of chemicals. More than half of the FDA-approved antibiotics and anticancer drugs discovered so far came from natural products. Some of the major challenges in accessing and studying these compounds include the difficulties in culturing and genetically manipulating the organisms of interest, and in inducing the expression of natural products. In addition, chemical synthesis of these compound is challenging, and many are not scalable. This study focuses on fungal polyketide biosynthesis. One approach to circumvent the difficulties in studying natural products is to heterologously express the gene clusters. Several compounds or their precursors have been successfully produced in this manner in recent years, with S. cerevisiae being developed as an excellent host to express fungal and plant gene clusters. Despite these successes, to date only 13 fungal clusters have been partially expressed in yeast, with most studies focusing only on early precursors of natural products. In this work, I build upon previous efforts and express the hypothemycin gene cluster in yeast. The core enzymes that make the chemical scaffold of hypothemycin are produced in our system. We will characterize chemical intermediates and function of the tailoring enzymes. In addition, we will alter hypothemycin tailoring genes and repurpose the pathway to make other fungal resorcyclic acid lactones (RALs), such as 5z-7-oxozeaenol. Efforts in completely reconstituting natural products pathways in heterologous hosts have been very limited, and our work will expand the knowledge in assembling natural products gene clusters in yeast, while allowing more manipulation of the pathway through tailoring enzymes.

397A. Chemical genomic profiling in *S. cerevisiae* and *S. pombe* to functionally annotate large compound collections. ***Sheena C. Li***[1,4], *Jeff Piotrowski*[2], *Raamesh Deshpande*[3], *Scott Simpkins*[3], *Justin Nelson*[3], *Jacqueline Barber*[1], *Minoru Yoshida*[1], *Chad L. Myers*[3], *Charles Boone*[1,4]. 1) RIKEN CSRS, Wako, Saitama, Japan; 2) GLBRC, University of Wisconsin-Madison; 3) University of Minnesota; 4) University of Toronto, Canada.
We have developed a robust method for annotating very large compound collections using a chemical genomics approach in *Saccharomyces cerevisiae*, thereby probing the physiological effects of compounds in a whole-cell assay. After compound treatment, quantification of the fitness defect associated with specific gene deletion mutants allows us to generate a chemical-genetic signature that can be highly predictive of compound mode of action.
To design a high-throughput pipeline using small amounts of precious compounds, we identified a diagnostic subset of deletion alleles and constructed these mutations in a drug-hypersensitive genetic background. Our assay is highly parallel because each deletion allele is marked with an oligonucleotide barcode, enabling us to use next-generation sequencing to measure the relative abundance of mutants within a pooled population. In total, we examined the chemical-genetic signatures of more than 14,000 compounds from the RIKEN Natural Product Depository and the NIH Open Chemical

Repository. After data normalization, compound targets were predicted by comparing chemical-genetic signatures with previously derived global synthetic lethal genetic interaction profiles.
Using the yeast genetic interaction network, we predicted high-confidence targets for ~800 compounds from the RIKEN NPDepo and created a functional map for these bioactive compounds. In addition, we generated chemical-genetic profiles for compounds from the NIH Open Chemical Repository, and are assessing how targets from this collection differ from the NPDepo. We are extending our analysis to another yeast, *Schizosaccharomyces pombe*, which offers the potential to probe pathways that are absent in *Saccharomyces cerevisiae*. By creating a large-scale chemical genomic network, our work captures a wide range of compound activities and reveals novel compound-target interactions.

398B. Genome-wide screening in yeast identifies genes involved in the localization of human poly (ADP-ribose) polymerase 1. ***Samuele Lodovichi***[1], *Giulia Rocchi*[1], *Marco La Ferla*[1], *Alberto Mercatanti*[1], *Tiziana Cervelli*[1], *Maria Adelaide Caligo*[2], *Alvaro Galli*[1]. 1) CNR, Pisa, Italy; 2) Azienda ospedaliera Santa Chiara, Pisa, Italy.
Inhibition of poly (ADP ribose)polymerase 1 (PARP1) is widely used in cancer therapy. The resistance to inhibitors of PARP which occurs in some patients has not yet been studied in depth. In this study, we overexpressed human PARP-1 gene in yeast and check the effect on growth and homologous recombination. We found that PARP-1 strongly inhibited yeast growth, but when yeast was exposed to the PARP-1 inhibitor 6(5-H) phenantridinone (PHE), it recovered from the growth suppression. Moreover, we demonstrated that PARP-1 reduced UV-induced homologous recombination. These results suggest that yeast has the same targets as mammalian cells. By screening for rapid growth on galactose medium immediately following transformation of the PARP-1 plasmid into a pool of genetically tagged deletion strains, we have identified 99 mutant strains that suppressed PARP-1 growth inhibition. The human homologous genes were found for a total of 41 yeast genes. Moreover, 20 genes that are involved in RNA processing, transcription, histone modification, chromatin remodeling, protein degradation or localization, may interfere with PARP-1 activity affecting the intracellular level or nuclear localization. We determined whether the PARP-1 protein level was altered in the strain which is deleted for the transcription regulator GAL3, the histone H1 gene HHO1, the HUL4 gene, the deubiquitination enzyme gene OTU1, the nuclear pore protein POM152 and the SNT1 that encodes for the Set3C subunit of the histone deacetylase complex . In the strains hho1, hul4, otu1, pom152 and stn1, the PARP-1 level was roughly the same as in the wild type. We showed that PARP-1 localized in the nucleus more in the snt1 than in the wild type strain; after UV radiation, PARP-1 localized in the nucleus more in hho1 and snt1 deletion strains than in the wild type indicating that these functions may have a role on regulating PARP-1 level and activity in the nucleus. Therefore, these protein activities may have a role in cancer therapy response and also in the response to PARP-1 inhibitors.

399C. Shared pHenotypes: Using a yeast genetic screen as a way to identify mammalian genes required for cellular survival during intracellular acidification. ***Jennifer A. McQueen***, *John Shin, Susan Li, Pamela Austin, Calvin Roskelley, Christopher Loewen.* Cellular and Physiological Sciences, Univ British Columbia, Vancouver, BR., BC, Canada.
Increased glycolysis is a hallmark of tumour cells and results in a large production of metabolic acids, which in turn causes intracellular acidification. Proton pumps such as the Na+/H+ exchanger 1 (NHE1), and the vacuolar H+ ATPase (V-ATPase) are upregulated in tumour cells reducing intracellular acidification and promoting tumour cell survival. These proton pumps are currently the focus of novel cancer drug research. Understanding how cells cope with intracellular acidification will lead to new targets for cancer therapeutics. The yeast, Saccharomyces cerevisiae, also experience intracellular acidification throughout the fermentative process. We have made use of a hypomorphic proton pump mutant *pma1-007* which when grown on acidic media caused intracellular acidification, to query for genes required for yeast cell survival during this stress. We performed a synthetic genetic array with non-essential yeast deletion set *pma1-007* double mutants on both neutral and acidic growth media. We identified 172 genes that when deleted caused a loss of growth in a pH and *pma1-007* dependent manner. We have begun work to determine if the homologous mammalian genes are required for cell survival during acid stress.

400A. The Effects of Omega-3-Fatty Acids on Intracellular inositol levels in Saccharomyces Cerevisiae. ***Marlene N Murray***, *Bomi Kim, Jee Yeon Lee.* Biology, Andrews University, Berrien Springs, MI.
Bipolar disorder is a severe and chronic debilitating mental disorder affecting 1-3% of the population. It is ranked sixth worldwide among all medical disorders in years of life lost to death or disability. Omega-3-fatty acids have been shown to relieve symptoms of bipolar disorder and are not associated with the negative side effects of lithium and valproate- two of the commonly used drugs for treating the disorder. However, the mechanism of action of omega-3-fatty acids remains unknown. In this study, the effects of the omega-3-fatty acid docosahexaenoic acid on growth and intracellular inositol levels was determined. We show that similar to valproate and lithium, this omega-3-fatty acid decreases growth of both wild type and inositol mutants. However, unlike valproate, it did not affect intracellular inositol levels.

401B. Making Genetic Suppressors for Yeast Genes Whose Orthologs are Involved in Human Retinitis Pigmentosa. ***Zahra NaghdiGheshlaghi***, *Jolanda van Leeuwen, Brenda Andrews, Charles Boone.* Donnelly Centre, University of

Toronto, Toronto, ON, Canada.
Some types of retinitis pigmentosa (RP), a dominantly inherited genetic disease that is characterized by progressive retinal degeneration and visual field defects, are caused by mutations in five genes that encode core spliceosomal proteins. Mutations in these five splicing proteins (Prpf3, Prpf6, Prpf8, Prpf31, and hBrr2) are the second most common cause of RP after mutations in rhodopsin. No drug treatment is currently available for RP and the molecular mechanisms underlying the disease are still unclear. Genetic suppression studies in model organisms have provided a powerful tool to uncover details of biological processes and to identify genetic relationships between two genes that might not be revealed using other genetic methods. Since the yeast homologs of the RP-related genes (*PRP3*, *PRP6*, *PRP31*, and *BRR2*) are essential in *Saccharomyces cerevisiae*, we are using temperature sensitive (ts) alleles to study their function. In this study, thirteen mutations in splicing genes known to cause RP in humans were introduced into the homologous yeast genes and the resulting yeast strains were tested for temperature sensitivity for their potential use in genetic suppression analysis.

402C. Genetic Analysis of the Warburg Effect in Yeast. ***Mobolanle Olayanju***, *James Hampsey, Michael Hampsey.* Department of Biochemistry and Molecular Biology, Rutgers University - R W Johnson Medical School, Piscataway, NJ.
We present evidence that the Warburg effect, defined by the dramatically enhanced metabolism of glucose to pyruvate, even in well-oxygenated cancer cells, is a consequence of mutations that enhance lipid biosynthesis at the expense of respiratory capacity. Specifically, mutations in either of two respiratory enzymes, pyruvate dehydrogenase (PDC) or -ketoglutarate dehydrogenase (KGDC), switch their substrate specificity from the 3-carbon -keto acid pyruvate, or the 5-carbon -keto acid -ketoglutarate, to the 4-carbon -keto acid oxaloacetate, resulting in aberrant synthesis of malonyl-CoA, the essential precursor of all fatty acids. To our knowledge, this activity, oxaloacetate dehydrogenase (OADC), has never been identified in eukaryotic cells. Our results offer a novel perspective on the Warburg effect: the reprogramming of energy metabolism in cancer cells would include mutational impairment of respiration to meet the fatty acid requirements of rapidly proliferating cells. As proof-of-principle, we have isolated suppressors of a yeast acc1cs cold-sensitive mutant that is deficient in malonyl-CoA synthesis as a consequence of defective AcCoA carboxylase activity (Acc1) at the restrictive temperature (16C). These mutants also suppress sensitivity to Soraphen A, a potent inhibitor of the Acc1 AcCoA carboxylase activity at normal temperature (30C). Remarkably, in addition to restoring growth at 16C and suppressing Soraphen toxicity, these spontaneous suppressors are respiratory defective as a result of the same nuclear mutations that bypass Acc1. We conclude that mutational inhibition of either PDC or KGDC impairs respiration, while OADC activity enhances fatty acid biosynthesis by bypassing AcCoA carboxylase, the enzyme that catalyzes the rate-limiting reaction in lipid biosynthesis. We suggest that this novel OADC activity is specific to cancer cells and represents a novel target for the development of chemotherapeutics.

403A. Discovering Novel Inhibitors of Deubiquitinases in vivo: Strategies using Budding Yeast. ***Natasha Pascoe***[1,2], *Michael Costanzo*[1,2], *Sachdev Sidhu*[1,2,3], *Charles Boone*[1,2,3]. 1) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Terrance Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; 3) Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.
Deubiquitinating enzymes (DUBs) are proteases that act as key regulators of the ubiquitin pathway by catalyzing the removal of ubiquitin from target substrates. Hence, these enzymes can affect a wide variety of cellular processes by influencing the localization, stability and function of their targets. Recent studies have implicated the misregulation of DUBs in numerous diseases. Importantly, the inhibition of DUB activity has emerged as a promising strategy for the treatment of several diseases, including cancer and neurodegenerative disorders. In spite of this, a paucity of specific and potent inhibitors aimed at DUB pathways has hindered attempts to manipulate them for therapeutic benefit. To address this issue, the Sidhu lab has developed a strategy that employs ubiquitin as a scaffold to generate highly specific and potent inhibitors of DUBs, which we call ubiquitin variants (Ubv). Using phage-display we have screened massively diverse and combinatorial libraries of these Ubvs to identify specific and potent inhibitors of DUBs. Although effective, in vitro screening methodologies pose certain limitations; for example, an inability to purify many DUBs has limited the applicability of these screening systems. Issues posed by our current screening systems be can circumvented through the use of in vivo screening techniques. Through a proof-of-principle study we have established the GAL4 based yeast two-hybrid system as a suitable platform for the detection of interactions between Ubvs and DUBs. Currently, we are working to adapt this system for high-throughput screening to allow for the development of novel Ubvs inhibitors of human DUBs. In conjunction with established screening methods, we will use our in vivo screens to discover inhibitors against the full panel of human DUBs. This work will make seminal contributions towards furthering our understanding of DUB function and alleviate a formidable bottleneck that obstructs the development of more powerful therapeutics aimed at the ubiquitin system.

404B. A connection between chromosome condensation/cohesion pathways and the toxic effects of the Huntington disease protein in a yeast model. ***Biranchi N. Patra***, *Scott Breslow, Jocelyn Wensel, Animesh Ray.* School of Applied Life Sciences, Keck Graduate Institute, Claremont, CA.

Huntingtons disease (HD) is caused by autosomal dominant alleles exhibiting expansion of a CAG repeat sequence within the first exon of the gene (HTT) encoding the Huntingtin protein. As a result of expansion of the CAG repeat over 40, the poly-glutamine (polyQ) repeat-containing protein functions aberrantly, which causes neuronal death in the striatum of the cerebral cortex, leading to cognitive decline and ultimately death. The age of onset of the disease is inversely proportional, with a variable penetrance, to the CAG repeat length above 40. Although many molecular targets of the toxic Huntingtin protein with expanded polyQ have been discovered, specific mechanisms for neurotoxicity that are causal to striatal neuron degeneration remain disputed, and genes affecting the age of onset remain largely undiscovered. An N-terminal fragment of Htt containing expanded polyQ(103) repeats is toxic in yeast, whereas the same fragment with a normal size polyQ(23) repeats is not. This model has been used over the past decade to search for genetic modifiers of Htt-mediated toxicity, in an effort to understand the molecular basis of HD. A recent previous study had identified deletion mutations *ume1* and *bna4* as suppressors of Htt mediated toxicity in yeast. Conversely, in a genome-wide dosage suppressor screen for a defect in chromosome condensation by the *smc2-8* mutation, we identified UME1, and BNA5 as suppressors. UME1 encodes a member of the histone deacetylase complex, and BNA4 and BNA5 encode two successive enzymes in the biosynthesis of NAD from kynurenine. These observations led us to explore whether there might be a mechanistic connection between Htt toxicity and the chromosome condensation-decondensation process. We discovered several genes involved in the latter process (SCC4, SMC1, SMC3, SMC4, YCS4, BRN1, and SPC24), and several genes involved in homologous recombination and/or genome integrity (DMC1, MEK1, MND1, UME1, HTA1, BUD16) as suppressors of the toxic Htt protein. These and other results enable us to connect BNA4/5 to a network of genes containing UME1, SCC2, and SMC2, and point to one possible mode of action of the mutant huntingtin protein involving the chromosome cohesion process. We speculate that interference by Htt with the normal sister-chromatid cohesion process might potentially increase somatic recombination at CAG repeats in the huntingtin gene, and thus could underlie the observed variable penetrance of high polyQ expansion alleles on disease onset age in human.

405C. Anti amyloidogenic potential of danshen derived chemicals and their analogs analysed by in vitro and in vivo yeast assay. ***Afsaneh Porzoor***[1], *Lynne J. Waddington*[2], *Helmut Hügel*[3], *Danilla Grando*[1], *Ian G. Macreadie*[1], *Joanne M. Caine*[2]. 1) Scool of Applied Sciences- Biosciences, RMIT University, Melbourne- Bundoora, VIC, Australia; 2) 2Materials Science and Engineering, CSIRO Preventative Health Flagship, 343 Royal Parade, Parkville, Victoria 3052, Australia; 3) School of Applied Sciences, Applied Chemistry, RMIT University, Melbourne, Victoria 3000, Australia.
The Chinese herbal medicine, danshen (Salvia miltiorrhiza) has been associated with many health benefits. Recent analysis has shown the presence of both lipid and water soluble active chemicals constituents in danshen which can inhibit the formation of amyloid beta fibril. In the present experiment a library of 21 chemicals (either isolated from danshen or synthesised) were screened for their anti amyloidogenic properties. Chemically synthesized amyloid beta (A42) in the presence of these chemicals was screened by Thioflavin T (ThT), immunoblotting (WO2 antibody), and transmission electron microscopy (TEM). Saccharomyces cerevisiae (BY4743) transformant with GFP fused to A42 were treated with the chemicals and were analysed by flow cytometry, microscopy and viable counts were performed. Flow cytometry screening of this S. cerevisiae transformants showed a significant increase in the fluorescence with sixteen chemicals (p 0.001) indicating an anti misfolding property. However, the analysis of the ThT assay for a period of 24 h only identified five chemicals with indicative anti-amyloidogenic propensity (p 0.0001). Confirmation of these results was performed using TEM and immunoblotting. This showed that selected compounds were capable of hindering or preventing fibril formation. Further, none of the conformers produced by the chemicals caused any toxicity to yeast cells while Rosmarinic acid, Caffeic acid and Gallic acid significantly increased the viability of these yeast cells. We have identified danshen active ingredients with anti amyloidogenic properties. This effect is not simply due to the presence of hydroxyl groups as previously reported. Our data also indicates that chemical isomers with similar hydroxyl groups do not necessarily have similar anti-amyloidogenic effects.

406A. A yeast synthetic genetic interaction screen predicts the importance of Plk1 for the viability of cancer cells over-expressing *CKS1b*. ***Robert J D Reid***[1], *Xing Du*[2], *Ivana Sunjevaric*[1], *Vinayak Rayannavar*[2], *John Dittmar*[1], *Matt Maurer*[2], *Rodney Rothstein*[1]. 1) Genetics & Development, Columbia University Medical Center, New York, NY; 2) Dept of Medicine, Columbia University Medical Center, New York, NY.
Cancer cells often over-express specific genes as a result of translocation or gene amplification. We used a high throughput screening protocol to mimic cancer-related over-expression in *Saccharomyces cerevisiae* mutants to identify genes whose functions become essential only when the cancer-related query gene is over-expressed. The resulting synthetic dosage lethal (SDL) interactions uncover conserved genes that may be targeted to kill cancer cells but leave normal cells unaffected. The *CKS1b* gene is located on chromosome 1q21 and is frequently amplified in breast, lung and liver cancers. Cks1b is a conserved regulatory subunit of cyclin-CDK complexes that functions at multiple stages of cell cycle progression. One of the pathways identified in the *CKS1* SDL screen controls a morphological checkpoint that leads to stabilization of the mitotic inhibitor Swe1, resulting in delayed mitosis when cell polarity is disrupted. Swe1 is a tyrosine kinase that inhibits the cyclin-dependent kinase (CDK) complex by phosphorylation of a conserved tyrosine-19

on Cdc28. We find that the SDL interaction between *CKS1* and the morphological checkpoint requires both Swe1 and the Cdc28 tyrosine-19 residue. Normal turnover of Swe1 occurs via a phosphorylation cascade, first by the cyclin-CDK complex, then by the conserved Polo-like kinase (Cdc5), and followed by SCF-mediated degradation. Since mutant alleles of *CDC5* were identified in the SDL screen, we investigated the effect of targeting human *PLK1* in breast cancers with varying expression of *CKS1b*. We first analyzed a published dataset of RNAi experiments to find *PLK1*-specific growth inhibition in 28 breast cancer cell lines. We then correlated these results to *CKS1b* expression levels from the Cancer Cell Line Encyclopedia to show that growth inhibition by *PLK1* knockdown correlates with increased *CKS1b*. Using shRNAs, we confirmed the correlation of between *CKS1b* expression and sensitivity to *PLK1* knockdown. Finally we engineered varying *CKS1b* expression in hMEC cells and showed that over-expression affects sensitivity to *PLK1* knockdown, showing a conserved genetic interaction.

407B. Efflux of the quinone methide maytenin through yeast cell *Saccharomyces cerevisiae* model. ***T.M. Souza-Moreira***[1], *V.A.F.F.M. Santos*[1], *S.R. Valentini*[2], *C.F. Zanelli*[2], *M. Furlan*[1]. 1) Organic Chemistry, Institute of Chemistry, UNESP, Araraquara, Sao Paulo, Brazil; 2) Biological Sciences, School of Pharmaceutical Sciences, UNESP, Araraquara, SP, Brazil.
Besides representing one of the most important eukaryotic model organism for the genetic and cell biology studies, *Saccharomyces cerevisiae* has proven to be an important in vivo system for the exploration of targets of biologically active small molecules and xenobiotics. More recently, not only the cell targets of the main and specific effects of small molecules are being studied in *S. cerevisiae*, but also their cell transporters, which control their flux across the cell. These studies made possible to uncover cell targets in yeast that are homologous to targets in other fungi and animals, providing essential clues in a more complex context of higher eukaryotes. Several small molecules had their mechanisms studied using *S. cerevisiae* and here we describe the study of the natural product maytenin. Maytenin, or tingenone, is a quinone methide triterpene from the barks of *Maytenus ilicifolia* (Celastraceae), a Brazilian species. Maytenin demonstrated to have antiinflammatory activity on different mammalian cell culture models and also to be an antifungal substance. We first examined the transport of maytenin across membrane transporters in *S. cerevisiae*. For the assay, the transporter knock-out cells were tested in 96-well plates prepared with twofold serial dilutions of maytenin (250 to 7.8 M). The plates were incubated at 30ºC and a solution of resazurin was added to determine cell viability after 48 h at 30ºC in a microtiter plate reader (SynergyH1, Biotek) at excitation and emission wavelengths of 530 and 590 nm, respectively. Curiously, *S. cerevisiae* wild type strain BY4741 is resistant to maytenin concentrations up to 250 M. On the other hand, the mutant *pdr5* did not grow in concentrations from 62.5 M to 250 M. *PDR5* codes for a multidrug resistance ATP-binding cassette (ABC) transporter and has been related to resistance to xenobiotic compounds and steroid transport. Therefore, it is likely that the resistance of the wild type to the concentrations of maytenin tested could be mainly due to the detoxification property of the efflux transporter Pdr5p in the plasma membrane. This result not only helps the understanding of the action of maytenin in cells, but also will make possible to study the mechanism of action of maytenin in *S. cerevisiae* using mutant and overexpression collections in a *pdr5* background.

408C. Small molecule inhibitors of human Tsg101. ***Katherine A Strynatka***, *Pak P Poon, Christopher R McMaster.* Dalhousie University, Halifax, Canada.
Human immunodeficiency virus type 1 (HIV-1) affects 35 million people worldwide. Current antiretroviral therapies, first introduced in the 1990s, slow down but do not completely suppress HIV replication. These drugs inhibit enzymes involved in viral replication (e.g. - nucleoside reverse transcriptase, integrase and protease) but the evolution of drug-resistant HIV-1 has become problematic and new options are needed. In addition to targeting viral enzymes, more recent research has also focused on protein-protein interactions required for viral replication. One such interaction involves the hijacking of host abscission machinery by HIV-1 for the release of virions from the infected cell. Specifically, the viral protein Gag interacts with the host protein Tsg101, a subunit of the Endosomal Sorting Complexes Required for Transport (ESCRT). In the cell, Tsg101 plays a role in vesicular transport and cytokinetic abscission. During the late stages of HIV-1 infection, Tsg101 interaction with Gag is required for release of HIV-1 from the cell; siRNA knockdown of Tsg101 impedes virion release. To target TSG101 for drug discovery, we expressed human Tsg101 heterologously in the budding yeast Saccharomyces cerevisiae. Expression of Tsg101 in yeast was found to be toxic to cell growth. In characterizing this phenotype, Snc1-GFP, a reporter of vesicular trafficking, was found to be mislocalized when Tsg101 was expressed. To find drugs that remedy the toxic effects of Tsg101 expression, a high-throughput small-molecule screen was performed and several compounds that restore yeast cell growth have been identified.

409A. Developing assays for pathogenic human variation via systematic testing of yeast/human complementation. ***Song Sun***[1,2,3,4,5], *Fan Yang*[1,2,3,4], *Guihong Tan*[1,2], *Michael Costanzo*[1,2], *Rose Oughtred*[6], *Jodi Hirschman*[6], *Chandra Theesfeld*[6], *Analyn Yu*[1,2,3,4], *Tanya Tyagi*[1,2,3,4], *Brenda Andrews*[1,2], *Nidhi Sahni*[7,8], *Song Yi*[7,8], *David Hill*[7,8], *Marc Vidal*[7,8], *Charlie Boone*[1,2], *Kara Dolinski*[6], *Frederick Roth*[1,2,3,4,7]. 1) Donnelly Centre, University of Toronto, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Department of Computer Science,

University of Toronto, Toronto, Ontario, Canada; 4) Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, Ontario, Canada; 5) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; 6) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, USA; 7) Center for Cancer Systems Biology (CCSB), Dana-Farber Cancer Institute, Boston, MA, USA; 8) Department of Genetics, Harvard Medical School, Boston, MA, USA.
It has become routine to identify all coding variants within an individual human genome. A pressing challenge is to functionally characterize these variants, particularly within disease-associated genes. To develop efficient functional assays for human genetic variants, we are systematically evaluating the ability of 'wild type' human disease genes to rescue yeast mutants. Initial efforts have focused on human genes with an essential yeast ortholog. Where no temperature-sensitive (TS) allele of the yeast gene was available, we generated TS alleles by systematic mutagenesis and screening. Rescue experiments identified 26 complementation relationships for the 142 tested orthologous pairs, while confirming 7 of the 11 known complementation relationships we curated from the literature. We exploited these functional complementation relationships as yeast-based functional assays to measure the impact of disease mutations from 10 human disease genes, with 17 of 22 disease alleles showing either complete or partial loss of complementation. To cast a wider net, we have developed a high-throughput barcode fusion genetics (BFG) technology potentially able to interrogate millions of human-to-yeast gene pairs for complementation in each experiment.

410B. Exploiting cancer-specific metabolism as a therapeutic strategy. ***L. H. Wong***, *G. Giaver, C. Nislow.* Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada.
Recent work has shown that the metabolic-specific state of the cancer cell is a potentially effective target. Unlike healthy cells, tumor cells exhibit accelerated glycolysis, even in the presence of oxygen. This phenomenon, known as the Warburg effect, is mechanistically similar to the Crabtree effect observed in budding yeast during fermentative growth. Cancer cells and budding yeast rely on similar enzymes to regulate glycolytic and fermentative fluxes in response to substrate availability making yeast a compelling model to exploit cancer-specific metabolism as a therapeutic strategy. The growth of budding yeast exhibits a diauxic shift characterized by an initial growth phase that depends on glucose fermentation followed by a second phase that engages oxidative phosphorylation in the presence of oxygen and upon glucose depletion. Using ~500 bioactive compounds profiled genome-wide in our laboratory (PMID: 24723613), we will re-screen compounds against a *S. cerevisiae* model that is proficient in fermentative and oxidative metabolisms. Compounds that alter the characteristic diauxic growth identify potential inhibitors of glycolysis, oxidative phosphorylation or the metabolic switch itself. Preliminary screens of established metabolic inhibitors Antimycin A and 2-deoxy-D-glucose have successfully abrogated cells diauxic growth. This proof-of-principle validates the use of the diauxic growth as a robust readout to identify metabolic-specific inhibitors. Candidate metabolic inhibitors will be validated for their *in vivo* mechanism of action using the HaploInsufficiency Profiling (HIP) and HOmozygous Profiling (HOP) yeast-based chemogenomic assays (PMID 14718668; 14718172; 18420932) and mammalian-based tests (PMID 22094260).

411C. Transcriptional regulation and protein complexes in budding yeast. ***Stacia R. Engel***, *Maria C. Costanzo, Kelley Paskov, J. Michael Cherry.* Dept Genetics, Stanford Univ, Stanford, CA.
The basis for much of our understanding of transcriptional regulation has been learned from the budding yeast *Saccharomyces cerevisiae*. Studies with yeast have also provided powerful insights into the diversity of protein complexes and the cellular pathways in which they are involved. We will present an update on new developments at the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/), the premier community resource for budding yeast. We have expanded the scope of SGD to include high quality manually curated information regarding transcriptional regulation and protein complexes. These new categories are provided in meaningful ways allowing data mining and discovery by integrating these data into this encyclopedic online resource. In addition to introducing our presentation of these newly curated data we will highlight other new developments, such as methods to display the depth of sequence variation within the many available genomes of *S. cerevisiae*. We already provide access to many of the strain genomes, in addition to the reference genome sequence of strain S288C. We will annotate and provide comparative analyses of these additional genomes, correlating sequence changes with variations in cellular phenotypes and protein function. SGD maintains these different datatypes, and distributes them to the scientific community via the web and file transfer. These expanded outreach efforts are part of our continuing mission to educate students, enable bench researchers and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

412A. TreeView 3.0: Visualization and Analysis of Two-Dimensional Genomic Data. ***Christopher Keil***, *Anastasia Baryshnikova*. Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.
Java TreeView is an open source software designed to visualize and analyze micro-array data. Developed over a decade ago, TreeView found wide application in many fields of genomics and is currently downloaded ~500 times per week. Despite its popularity, TreeView has not been updated since 2004 and has accumulated numerous requests for updates and new features. Here we report the release of a new TreeView 3.0, which builds upon the original Java TreeView and

expands its data visualization capabilities. Features of the new TreeView 3.0 include a reworked graphical user interface for improved workflow, enhanced customization, as well as seamless navigation through data sets. In addition, TreeView 3.0 implements a clustering feature that will eliminate the requirement of additional clustering software and streamline data analysis. We are working towards maintaining a continuous user experience while improving software performance and efficiency, specifically in relation to large input data sets. New releases, updates, feature requests and additional information are available at www.baryshnikova-lab.org/treeview/.

413B. WITHDRAWN

414C. Tyre: An *in Silico* Simulator of Population Dynamics. ***Jill N Wright***[1], *Jessica Stilwell*[2], *Christopher Zahner*[1], *Hui Hua*[1], *Brandt L Schneider*[1]. 1) Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX; 2) Texas Tech University, Howard Hughes Medical Institute, Lubbock, TX.
Appropriate coordination of growth and proliferation is vital for proper development. Cell growth in the absence of proliferation is essential for oogenesis. However, inappropriate excessive growth can result in carcinogenesis, while lack of growth can hinder wound healing and tissue regeneration. Yeast is an excellent model for studying growth control as the mechanisms involved are required for cell size homeostasis. In yeast, while the size distributions of cell populations are very reproducible, individual cell size can vary tremendously within the population. In addition, cell size is genetically programmed and modulated by environmental conditions. However the mechanisms responsible for cell size homeostasis are not well understood. Currently, two hypotheses have been proposed to explain cell size homeostasis: 1. Commitment to cell cycle progression is regulated by a time dependent step; 2. Commitment to cell cycle progression is regulated by a cell size dependent step. To test these hypotheses, we have developed a computer simulator (TYRE) to model population dynamics. Results indicate that the sizer model is better at modeling homeostasis. Furthermore, *in silico* modeling suggests that exponential cell growth models (e.g. the rate at which cells add mass) most closely match observed population dynamics. TYRE provides insight into the mechanisms of yeast cell growth by simulating potential interactions between growth and proliferation. Since many pathways involved in cell size homeostasis in yeast are evolutionarily conserved, results in this study should also help elucidate the mechanisms involved in growth control in both yeast and higher eukaryotes.

415A. Balony: a software package for analysis of data generated by synthetic genetic array experiments. ***Barry Young***, *Christopher Loewen.* Dept Cell Phys Sci, Life Sci Inst, Vancouver, BC, Canada.
Synthetic Genetic Array (SGA) analysis is a procedure which has been developed to allow the systematic examination of large numbers of double mutants in the yeast *Saccharomyces cerevisiae*. The aim of these experiments is to identify genetic interactions between pairs of genes. These experiments generate a number of images of ordered arrays of yeast colonies which must be analyzed in order to quantify the extent of the genetic interactions. We have designed software that is able to analyze virtually any image of regularly arrayed colonies and allows the user significant flexibility over the analysis procedure.
Balony is freely available software which enables the extraction of quantitative data from array-based genetic screens. The program follows a multi-step process, beginning with the optional preparation of plate images from single or composite images. Next, the colonies are identified on a plate and the pixel area of each is measured. This is followed by a scoring module which normalizes data and pairs control and experimental data files. The final step is analysis of the scored data, where the strength and reproducibility of genetic interactions can be visualized and cross-referenced with information on each gene to provide biological insights into the results of the screen.
Analysis of SGA screens with *Balony* can be either automated or highly interactive, enabling the user to customize the process to their specific needs. Quantitative data can be extracted at each stage for external analysis if required. Beyond SGA, this software can be used for analyzing many types of plate-based high-throughput screens.
Further information and downloads are available at http://balony.googlecode.com.

416B. Profiling the RNA maturation landscape in yeast. *Alexander Ratushny*[1,2], *Marlene Oeffinger*[4], *Wei-Ming Chen*[2], *Karen Wei*[4], *Peter Fridy*[3], *Richard Rogers*[2], *Ramsey Saleem*[1,2], *Garrett Poshusta*[1], *Michael Rout*[3], ***John Aitchison***[1,2]. 1) Systems Biology, Institute for Systems Biology, Seattle, WA, USA; 2) Seattle Biomedical Research Institute, Seattle, WA, USA; 3) Rockefeller University, New York, NY, USA; 4) Institut de recherches cliniques de Montréal, Montréal, Québec, Canada.
All RNAs are transcribed and assembled into ribonucleoprotein (RNP) complexes and these diverse and dynamic complexes play crucial roles in RNA transcription, processing, nucleocytoplasmic export, translation, and decay. While many RNP components are known, given the vast amount of RNA Pol II transcribed RNAs alone, and the relatively small number of known RNA maturation factors, several open questions remain; how much overlap between subsets of proteins exists for different Pol II transcripts? Are all mRNAs processed along the same pathway, or are there distinctions for different classes of transcripts? What is the intra-complex landscape, the direct interaction, within these RNPs? To obtain a

comprehensive view on mRNP maturation, we performed a quantitative analysis of yeast RNP complexes at the system level. In this study, thirty five proteins from different stages of the mRNA maturation pathway (from transcription through different processing steps to export and degradation of faulty complexes) were selected for analysis. The RNP complexes of the selected protein A-tagged proteins have been isolated under different conditions and from different genetic backgrounds (i.e., conditional and deletion mutants). The bait-associated RNAs and proteins were isolated by single-step affinity purification and analyzed by RNA-seq and mass spectrometry, respectively. This systems biology approach detects many thousands of high quality and reproducible protein-protein and protein-RNA interactions as well as complex network rewiring events of multiple targeted RNP composites under conditional perturbations. The systematic functional and network analysis of this unprecedented data set reveals hundreds of previously uncharacterized protein-protein and protein-RNA interactions and novel molecular control mechanisms of the coordination of cellular processes that are now experimentally validated in a targeted manner.

417C. Cin5, Gln3, Hmo1, and Zap1 Contribute to the Gene Regulatory Network Controlling the Cold Shock Response in *Saccharomyces cerevisiae*. ***Kam D. Dahlquist***[1], *Ben G. Fitzpatrick*[2], *Cybele Arsan*[1], *Wesley T. Citti*[1], *Kevin C. Entzminger*[1], *Andrew F. Herman*[1], *Lauren N. Kubeck*[1], *Stephanie D. Kuelbs*[2], *Heather King*[1], *Elizabeth M. Liu*[1], *Matthew Mejia*[1], *Kenny R. Rodriguez*[1], *Nicholas A. Rohacz*[1], *Olivia S. Sakhon*[1], *Katrina Sherbina*[2], *Alondra J. Vega*[2]. 1) Biol, Loyola Marymount Univ, Los Angeles, CA; 2) Math, Loyola Marymount Univ, Los Angeles, CA.
The global transcriptional response of budding yeast, *Saccharomyces cerevisiae*, to cold shock and subsequent recovery was measured using DNA microarrays. Yeast cells were grown to early log phase at 30C, then shifted to 13C for 60 minutes, and then shifted back to 30C for another 60 minutes. Samples were collected before cold shock, after 15, 30, and 60 minutes of cold shock, and after 30 and 60 minutes of recovery at 30C. Clusters of genes involved in ribosome biogenesis, zinc ion homeostasis, and hexose transport were found to be up-regulated upon cold shock and down-regulated upon recovery. Genes involved in protein and vesicle transport, the endomembrane system, and negative regulation of nitrogen compounds showed the opposite pattern of expression. To determine which transcription factors were responsible for these changes in expression, the growth rates of transcription factor deletion strains were determined at 30 and 13C in liquid media. The *gln3* and *hmo1* strains had slower doubling times at 13C than the corresponding BY4741 parental strain, while the *cin5* and *zap1* strains did not. The same cold shock and recovery microarray experiments were then performed on these four deletion strains. Statistical analysis using a general linear model revealed that approximately 8% of genes showed a significantly different pattern of expression at p 0.05 between the wild type and each deletion strain. Finally, we used these microarray data to estimate parameters for an ordinary differential equations model of a 21-gene regulatory network controlling the cold shock response. The equation for each gene includes a production rate, a degradation rate, weights that denote the influence of transcriptional regulators upon the gene, and a term for the threshold of expression. Model predictions fit the data well within two standard deviations of the experimental error and revealed the relative influence of these transcription factors on the network dynamics. This work was supported by NSF award 0921038 (KDD, BGF, CA, AFH, LNK, NAR, KS, and AJV).

418A. Dissecting the Regulation of the Yeast Pleiotropic Drug Response. ***Colin Harvey***, *Ulrich Schlecht, Sundari Suresh, Robert St. Onge, Ronald Davis, Maureen Hillenmeyer.* Stanford Genome Technology Center, Stanford University, Palo Alto, CA.
The ability to yeast to thrive in the presence of a variety of diverse chemical agents is primarily the result of a network of ABC transporters collectively termed the pleiotropic drug response (PDR). In addition to its essential role in yeast biology, the PDR also serves as an important model for human multidrug resistance. While many of the transporters involved in this response are well characterized, the regulatory cascade by which their expression is upregulated in the presence of a diverse panel of drugs has yet to be fully determined. Here we present work toward the dissection of this regulatory network through the construction of plasmids consisting of the HIS3 gene driven by the promoters of a series of PDR genes. These biosensor plasmids were then transformed into the genome-wide homozygous diploid deletion collection. The relative growth-rates of individual deletion strains in these pools in the presence of a HIS3 inhibitor and a variety of compounds known to induce the PDR provides valuable, genome-wide insight into the regulation of this complex transporter network.

419B. Transferability of protein-protein interactions and protein function between closely related species. ***Hsueh-lui Ho***[1], *Maxime Huvet*[2], *Michael Stumpf*[2], *Ken Haynes*[1]. 1) Biosciences, 3rd floor Geoffrey Pope Building, Exeter University, Exeter, Devon, EX4 4QD United Kingdom; 2) Theoretical System Biology, Imperial College London, London, United Kingdom.
Mapping and understanding of protein interaction networks (PINs) are part of the key to unlocking the link between genotypes and phenotypes. Due to technical limitations (time and money), protein-protein interactions (PPIs) have been experimentally identified in only a few species. To further our knowledge of PIN evolution and the relation between PINs and phenotypes observed, we examined the PPIs in two closely related yeast species; the non-pathogen model organism

Saccharomyces cerevisiae and the opportunistic pathogen *Candida glabrata*. A common assumption made between species is that orthologues share a similar function, protein-protein interaction (PPI) profile, and genetic interactions. Here we show that this is not true for all orthologues, even between two closely related species. Firstly, we examined the simple assumption that the higher the conservation of Protein A and Protein B pairs between *C. glabrata* and *S. cerevisiae*, the greater the conservation between the two networks. Initial interactions for Yeast-2-Hybrid (Y2H) screening of *C. glabrata* proteins were selected based on orthologue similarity, how well PPI covariates are associated to reported *S. cerevisiae* interactions, and whether they form part of the same system. As expected, we show that transferability from protein affinity networks to Y2H is poor but that transferability between Y2H networks in yeast is relatively high. Our results strongly indicate that conservation of a PPI between the two species is not linked to the level of protein sequence similarity. Next, we examined the highly conserved Glycerol-3-phosphate dehydrogenase (Gpd1/2) enzymes in *S. cerevisiae* and *C. glabrata* and show not only that the PPI profiles of the two species differ, but that there are functional differences in response to osmotic stress. Despite the close phylogenetic proximity between the two species and the high number of shared orthologues, our results confirm that great care should be taken when transferring PPIs between species and that not all orthologues perform the same function in different species.

420C. Measuring changes in genetic interactions over environments using iSeq. ***Mia Jaffe***[1], *Gavin Sherlock*[1], *Sasha F Levy*[2]. 1) Dept of Genetics, Stanford University, Stanford, CA; 2) Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY.
Two genes are said to interact when the fitness of a double mutant deviates from the multiplicative fitness of the two corresponding single mutants. Mapping interactions can lead to the discovery of new gene functions and a better understanding of the link between genotype and phenotype. Since the creation of the yeast deletion collection, over the last decade, enormous progress has been made in understanding genetic interactions. Approximately 75% of all possible pairwise interactions have been assayed on plates with rich medium. However, little is known about how, or even if, these interactions change with environmenta problem not easily addressed with current methods. iSeq is a powerful new method to assay the fitness of single and double mutants using next-gen sequencing at several time points over pooled growth. iSeq relies on a technology whereby two unique barcodes, each associated with a unique parent, are brought together to a common genomic location in vivo and en masse. Components of this technology have been functionally verified in initial experiments. Events that bring two barcodes together occur at a non-limiting frequency (20-50% of cells), and a subset has been verified by sequencing. Currently, interactions between nine genes are being measured, compared to published data, and measured again in different environments. If successful, iSeq will be scaled up, and the genetic interaction data generated will have a high impact on our understanding of changes in genetic buffering in different environments.

421A. Extensive inbound and feedback signal integration by Protein Kinase A. ***Christian Landry***[1], *Marie Filteau*[1], *Guillaume Diss*[1], *Francisco Torres-Quiroz*[1], *Alexandre Dube*[1], *Isabelle Gagnon-Arsenault*[1], *Andree-Eve Chretien*[1], *Ugo Dionne*[2], *Anne-Lise Steunou*[2], *Andrea Schrafft*[3], *Jacques Cote*[2], *Nicolas Bisson*[2], *Eduard Stefan*[3]. 1) Département de Biologie, PROTEO and Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Québec, G1V 0A6, Canada; 2) Département de Biologie Moléculaire, Biochimie Médicale et Pathologie, et Centre de Recherche sur le Cancer, Université Laval, Québec, Québec, G1V 0A6, Canada; 3) University of Innsbruck, Institute of Biochemistry and Center for Molecular Biosciences Innsbruck (CMBI), A-6020 Innsbruck, Austria.
Master regulators coordinate cellular processes and control homeostasis in eukaryotic cells. While the outbound signals from Protein Kinase A (PKA) directed to processes such as metabolism, growth and aging have been well charted, what regulates this master regulator remains to be systematically identified. Using a yeast PKA reporter assay to map regulators genome-wide, we identified 500 genes that are connected directly or indirectly to PKA regulation and that span multiple processes, including the antagonistic autophagy and methionine biosynthesis pathways. We find that PKA regulation by lysine acetylation, which is conserved between yeast and humans, regulates both carbohydrate storage and aging. The diversity of inbound signals to the PKA demonstrates its central position in orchestrating cellular processes.

422B. Genome-scale analyses of *Saccharomyces cerevisiae* strains evolved for bio-ethanol production under aerobic and anaerobic growth conditions. ***Kevin S. Myers***[1,2], *Nicholas M. Riley*[3,4], *Trey K. Sato*[2], *Joshua J. Coon*[3,4,5], *Audrey P. Gasch*[1,2]. 1) Laboratory of Genetics, UW-Madison, Madison, WI; 2) Great Lakes Bioenergy Research Center, UW-Madison, Madison, WI; 3) Department of Chemistry, UW-Madison, Madison, WI; 4) Genome Center, UW-Madison, Madison, WI; 5) Department of Biomolecular Chemistry, UW-Madison, Madison, WI.
Biofuels produced by microbes, including *Saccharomyces cerevisiae*, have the potential to be part of a non-petroleum based energy system. Production of ethanol from non-food biomass, such as corn stover, would provide an important advance, especially because corn stover contains high concentrations of glucose and xylose. *S. cerevisiae* cannot naturally ferment xylose, for reasons that are not understood, preventing it from using a third of the potential carbon in corn stover biomass. To this end, an engineered *S. cerevisiae* strain containing bacterial xylose isomerase was subjected to direct evolution for over 200 generations: strain Y127 was evolved from a stress-tolerant wild strain for aerobic xylose

fermentation. This strain cannot ferment xylose anaerobically, and therefore Y127 was subjected to cycles of anaerobic evolution, producing strain Y128 that ferments xylose anaerobically in corn stover hydrolysate. Whole-genome sequencing identified responsible mutations in the evolved strains, but the physiological effect of these mutations remains unclear. To better understand the physiology of these strains, we performed genome-scale analyses to study differences in gene expression (RNA-seq), protein levels (label-free quantitative mass-spec proteomic data) and protein phosphorylation (isobaric tagging coupled to mass-spec identification) on the parental strain and the two evolved strains, in the presence of glucose or xylose and under aerobic or anaerobic growth conditions. Computational comparison of these data implicates several biochemical pathways and physiological responses specific to evolved strains, suggesting potential metabolic bottlenecks in the parent that have been partially overcome. These results allow us to better understand xylose fermentation and will aid in further optimization of *S. cerevisiae* for biofuel production.

423C. A gene network model of cellular aging and its applications. ***Hong Qin***. Biology, Spelman College, Atlanta, GA.
Why would a genotypically homogeneous population of cells live to different ages? We propose a mathematical model of cellular aging based on gene interaction network. This model network is made of only non-aging components, and interactions among genes are inherently stochastic. Death of a cell occurs in the model when an essential gene loses all of its interactions. The key characteristic of aging, the exponential increase of mortality rate over time, can arise from this model network with non-aging components. Hence, cellular aging is an emergent property of this model network. The model predicts that the rate of aging, defined by the Gompertz coefficient, is proportional to the number of active interactions per gene and that stochastic heterogeneity is an important factor in shaping the dynamics of the aging process. Hence, the Gompertz parameter is a proxy of network robustness. We then show how this model can be applied to study yeast aging using the empirical yeast gene network. We will also present results on how aging is influenced network configuration and how limiting gene interactions can be used to compare competing hypotheses on cellular aging.

424A. Genetic pathways involved in response to the phenol-based compounds bisphenol-A (BPA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in Saccharomyces cerevisiae. ***Shravya L. Raju***[1], *Julia Levy*[2], *Elizabeth Martin*[3], *Mia Pecora*[1], *Gretchen Edwalds-Gilbert*[1,2,3]. 1) Scripps College, Claremont, CA; 2) Pitzer College, Claremont, CA; 3) Claremont McKenna College, Claremont, CA.
Phenolic compounds exist in abundance in the environment as the product of natural and synthetic processes. Phenols produced from chemical, petrol, and pharmaceutical processes can be harmful, stress-inducing exotoxins. Bisphenol-A (BPA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are phenol-based compounds found in polycarbonate plastics and food preservatives and therefore have a particularly high degree of human exposure. Exposure to these compounds has been associated with numerous diseases, yet their mechanisms of action are not well understood. A quantitative evaluation of growth of yeast deletion strains was used to identify the genes that modulate response to phenol stress induced by BPA, BHA, and BHT in the model organism Saccharomyces cerevisiae. No strains showed growth impairment in the presence of BHT. Twelve genes were identified as essential for growth in the presence of both BPA and BHA. pep5 and vph2 are integral to vacuolar ATPase function, erg2 and erg24 are involved in the ergosterol biosynthesis pathway, and ref2, rsc1, bdf1, tps2, hpr1, and snf12 are important in chromatin remodeling and transcription processes. Additional genes required for growth and resistant genes were identified that were specific to BPA or BHA. We validated the role of the vacuolar ATP-synthase complex in response to BHA, and found that V-ATPase activity increases in wild type yeast in the presence of the chemical. Mutants deleted for components of the V-ATPase complex show impaired growth in BHA-containing media. We examined the phenotypes of erg strains and found that BPA induces the unfolded protein response (UPR) in these strains, and BHT induces the UPR in wt yeast. Many of the genes and pathways identified in S. cerevisiae have orthologs in humans that may modulate exposure to toxic phenols in a similar manner and could provide insight into the pathways involved in phenol exposure related disease.

425B. Widespread changes in the yeast protein interaction network in response to diverse environmental cues. ***U. Schlecht***[1], *J. Smith*[1], *A. Celaj*[2], *S. Suresh*[1], *M. Miranda*[1], *R.W. Davis*[1], *F. Roth*[2], *R.P. St.Onge*[1]. 1) Biochemistry, Stanford University, Palo Alto, CA; 2) Donnelly Centre, University of Toronto, Toronto, Ontario.
Changes in protein-protein interactions that occur in response to environmental cues are difficult to uncover and poorly characterized to date. Here we describe a yeast-based assay that allows many binary protein interactions to be assessed in parallel and under various conditions. This method combines molecular bar-coding and tag array technology with the mDHFR (murine Dihydrofolate Reductase)-based Protein-fragment Complementation Assay (PCA). More than one thousand PCA strains, each representing a unique binary protein complex, were tagged with molecular bar-codes, pooled, and then interrogated against a panel of diverse environmental stress conditions (such as high salt and DNA damage). We find widespread changes in the yeast protein interaction network (PIN) in response to these environmental cues many of which can be explained by transcriptional changes in the underlying genes. We also explored the effects of targeting individual nodes in the PIN using a CRISPR-Cas9 mediated transcription system.

426C. TheCellMap.org: storing and visualizing genetic interactions in S. cerevisiae. ***Matej Usaj***[1], *Michael Costanzo*[1], *Chad L Myers*[2], *Brenda Andrews*[1], *Charles Boone*[1], *Anastasia Baryshnikova*[3]. 1) Donnelly CCBR, University of Toronto, Toronto, Ontario, Canada; 2) Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN, USA; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA.
Providing access to quantitative genomic data is key to ensure large-scale data validation and promote new discoveries. Here we report the launch of a web application, theCellMap.org, that serves as the central repository for quantitative genetic interaction data for Saccharomyces cerevisiae produced by systematic Synthetic Genetic Array (SGA) experiments in the Boone/Andrews lab. TheCellMap.org provides a set of fundamental tools for analyzing genetic interaction data. In particular, theCellMap.org allows a user to easily visualize, explore and functionally annotate genetic interactions as well as extract and re-organize sub-networks using data-driven or annotation-driven network layouts in an intuitive and interactive manner.

427A. Unraveling protein network evolution through whole-proteome cross-species interactome mapping. ***Haiyuan Yu***. Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.
The fission yeast Schizosaccharomyces pombe has more metazoan-like features than the budding yeast Saccharomyces cerevisiae with similarly facile genetics. Yet, it is significantly under-studied with little functional genomic information available. Here, we screened the whole fission yeast proteome three times (75 million protein pairs) to generate the first high-coverage high-quality binary interactome network for S. pombe, FissionNet, comprising ~2500 interactions among ~1300 proteins. We further perform the first whole-proteome well-controlled cross-species interactome mapping between the two yeast species, S. cerevisiae and S. pombe. We analyzed conservation patterns of different pathways and found significant variations in the extent to which different biological functions are conserved across the two yeasts. We find that intra-pathway interactions are well conserved between the two species, but considerable inter-pathway interactions have been rewired. Furthermore, we find that conserved interactions tend to be stable, and rewired ones are more likely to be transient. Finally, our FissionNet identifies many novel factors in various pathways, from those that budding yeast does not possess but fission yeast shares with mammals (such as RNAi) to those that are well conserved between both species (such as MAPK pathways).

428B. Functional Characterisation of *Candida glabrata* Open Reading Frames with no Orthologue in *Saccharomyces cerevisiae*. ***Lauren C Ames***[1], *Jane Usher*[1], *Ilias Kounatidis*[2], *Petros Ligoxygakis*[2], *Ken Haynes*[1]. 1) Biosciences, University of Exeter, United Kingdom; 2) Department of Biochemistry, University of Oxford, UK.
Candida glabrata is a significant and increasingly common pathogen of humans yet its mechanism of virulence remains unclear. Comparative genomic studies revealed that *C. glabrata* is more closely related to the non-pathogenic yeast *Saccharomyces cerevisiae* and that both these genomes are distinct from *C. albicans*. In order to explore *C. glabrata* virulence attributes, *C. glabrata* ORFs with no orthologue in *S. cerevisiae* were studied since these ORFs may have accompanied the adaptation of *C. glabrata* to the human host.
Reciprocal best hit searches identified *C. glabrata* ORFs with no *S. cerevisiae* orthologue. A barcoded deletion library targeting 65 *C. glabrata*-specific ORFs was constructed. To functionally characterise the deletion library, mutants were tested for fitness and phenotypically screened to identify gene products required for growth in response to biologically relevant stresses. As such, novel phenotypes associated with the deletion of previously uncharacterised ORFs were uncovered. Mutants were also tested for infection-related properties including biofilm formation, antifungal agent susceptibility for virulence in a *Drosophila melanogaster* infection model, resulting in the identification of two ORFs, encoding a putative oxidoreductase and an ORF with no bioinformatically recognisable domains, which were required for virulence.
ORFs with notable phenotypes were taken forward for further characterisation. An adapted genome-wide synthetic genetic interaction approach was used to create genetic interaction networks for *C. glabrata* ORFs expressed in *S. cerevisiae*. Genetic interaction analysis of a putative *C. glabrata* chromatin remodeler *CAGL0D05434g* revealed a role for this ORF in metal ion homeostasis and DNA damage repair, which was further confirmed in phenotypic screens. Genetic interaction profiling for an oxidoreductase encoded by *CAGL0K05687g* was used to reveal mechanisms by which this ORF may be required for virulence.

429C. Comparative genomics and transcriptomics of the industrial yeast species *Dekkera* (*Brettanomyces*) *bruxellensis*. *Anthony Borneman*[1], *Lucy Joseph*[2], *Toni Cordente*[1], *Robyn Kievit*[1], *Ryan Zeppel*[1], *Warren Albertin*[3], *Isabelle Masneuf-Pomarede*[3], *Linda Bisson*[2], ***Chris Curtin***[1]. 1) Australian Wine Research Institute, Urrbrae, SA 5064, South Australia, Australia; 2) Department of Viticulture and Enology, University of California, Davis, 595 Hilgard Lane, Davis, CA 95616; 3) Univ. de Bordeaux, ISVV, EA 4577, Unité de recherche Œnologie, F-33140 Villenave 13 d'Ornon, France.
Dekkera bruxellensis, like its wine yeast counterpart *Saccharomyces cerevisiae*, is intrinsically linked with industrial fermentations. In wine, *D. bruxellensis* has what are generally considered negative influences on wine quality, whereas for some styles of beer it is an essential contributor. *D. bruxellensis* also plays a role in bioethanol fermentation - sometimes

beneficial, but in other systems detracting from production efficiency by outcompeting *S. cerevisiae*. We previously investigated the level of inter-strain variation that is present within this economically important species, by comparing the genomes of four diverse *D. bruxellensis* isolates. Two of these genomes were predicted to be triploid, comprising a core diploid set of chromosomes and a third divergent haploid set, reminiscent of allotriploids within the *Saccharomyces sensu-stricto*. Re-sequencing of a further 38 isolates from around the world revealed that triploidy is a relatively common status for *D. bruxellensis*. Our data suggest at least five independent 'hybridisation' events have occurred to generate these triploid lineages that now populate brewing, winemaking and soft-drink related niches. It is unclear what fitness benefits triploidy has conferred, although one lineage exhibits greater tolerance to the common preservative sulfite. In order to better understand the basis of this phenotypic advantage, comparative transcriptomics was performed for two strains during growth in model-wine conditions and when exposed to sulfite. Similar to *S. cerevisiae*, relatively few transcripts were differentially expressed in response to sulfite. A clear case of allele-specific expression (ASE) for a putative sulfite pump encoding gene was detected, and the global instance of ASE across the triploid genome examined to infer genes for which the divergent allele may be beneficial.

430A. Assembling the *Schizosaccharomyces kambucha* genome and the dynamic transposon landscape of fission yeasts. ***Michael Eickbush***[1], *Sarah Zanders*[1], *Gerry Smith*[1], *Harmit Malik*[1,2]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Research Institution, Chevy Chase, MD.
In the fission yeast *Schizosaccharomyces pombe* (*Sp*), the positions of all transposable elements (TE) and their remnants (known as LTR sequences) have been annotated. To better understand how TE landscapes change over time and how TEs affect genome organization, we used a combination of next-generation sequencing technologies (Illumina paired-end 50 base pair reads, and longer Pac-bio reads), PCR, and Southern blot hybridizations to largly assemble the genome of *Schizosaccharomyces kambucha* (*Sk*), a fission yeast very closely related to *Sp*. Ignoring repetitive elements, the *Sp* and *Sk* genomes are nearly identical (99.5%) at the sequence level. Despite the low level of divergence between the two species, we found that the TE landscape was dramatically different between the two. *Sk* contains two types of transposons, whereas the reference *Sp* genome contains only one. In addition, the *Sk* genome has had twice as many transposon insertion sites as *Sp*. The majority of transposons in *Sk* have been maintained as full length, while most of the transposons have been lost from *Sp*. This leads *Sk* to contain nearly five-times more full length TEs and no full-length transposons are shared between the two species. We also found that recombination between TEs in *Sk* caused a reciprocal chromosome translocation and two chromosome inversions within this species. Chromosome rearrangements such as these contribute to hybrid infertility. Our genome assembly has revealed that TEs are primary drivers of genome evolution between *Sp* and *Sk*.

431B. Evolutionary genomics of ecological speciation in Saccharomyces paradoxus. *Jean-Baptiste Leducq, Lou Nielly-Thibaut, Guillaume Charron,* ***Christian Landry***. Département de Biologie, Université Laval, Quebec, QC, Canada.
Understanding the genomics bases of species formation in relation with their ecological causes and consequences is one of the major challenges in evolutionary biology. Saccharomyces cerevisiae and its closely related species have been a prime model in the study of the genomics bases of species divergence but our understanding of the ecological context in which speciation takes place in these genus is extremely limited. In order to fill this gap, we investigate the genomic architecture and ecological context of an incipient speciation event in the wild budding yeast Saccharomyces paradoxus. We identified two partially reproductively isolated American lineages of S. paradoxus that show strong divergence in metabolism for carbon and nitrogen sources, and adaptations to climatic conditions, consistent with their ecological specialization. The extent of reproductive isolation between the two lineages correlates with karyotypic changes, suggesting a role for chromosomal changes in this speciation event. In order to map genomic and genetic changes to the divergent ecological traits, we sequenced the genome of 150 strains representative of these two lineages. Using de novo assembly, we identified changes in gene copy number and chromosomal rearrangements that occurred during lineage divergence, some of which may correspond to adaptive changes. Some of the chromosomal rearrangements correspond to introgression events, indicating that recent and ancient hybridization events have occurred between these lineages. Finally, we are using the panel of sequenced strains to map phenotypic changes to genetic variation and divergence using high-throughput phenotyping and association mapping. Our results demonstrate the power of using budding yeasts as model systems in ecological genomics investigations and illustrate how genome analyses can illuminate the ecological determinants of speciation events in this model genus.

432C. Comparative Genomics of Yeast Genome Conformation and Functional Annotation by Multiplexed Hi-C. ***Ivan Liachko****, Joshua Burton, Jay Shendure, Maitreya Dunham.* Genome Sciences, University of Washington, Seattle, WA.
The three-dimensional organization of chromosomes within the cell relates to fundamental processes such as transcriptional regulation and chromosome replication and segregation. However, little is known about the relationship of nuclear organization and evolution due to a dearth of data on non-canonical model organisms.

To model the 3D structure of a genome, chromosome conformation capture techniques such as Hi-C are used to measure

long-range interactions of DNA molecules. These tools employ crosslinking of chromatin in intact cells followed by intra-molecular ligation joining DNA fragments that were physically nearby at the time of crosslink. Subsequent deep sequencing of these DNA junctions generates a genome-wide contact probability map that allows the reconstruction of high-quality genome assemblies and 3D modeling of genomic conformation within a cell. These methods also preserve the cellular origin of each DNA fragment and its interacting partner allowing for deconvolution of multi-chromosome genomes from a mixed population of organisms.

We have used Hi-C on mixed populations of yeast to generate contact probability maps for over 20 diverse yeast species including members of *Saccharomyces*, *Schizosaccharomyces*, *Lachancea*, *Kluyveromyces*, *Ashbya*, *Pichia*, and other genera. These maps allow for comparative genomic study of chromosomal organization across species that fall in wide ranges of evolutionary distance. We have also been able to use this data to annotate functional features of genomes, such as centromeres, which can be cloned to produce ARS/CEN plasmids in diverse yeasts. Additionally, our data has allowed us to produce improved genome assemblies for several sequenced yeasts by utilizing the enrichment of Hi-C signal at close intrachromosomal loci as an improved means of genome scaffolding.

433A. Identifying biological pathway targets for lipid production in Yarrowia lipolytica. ***Kyle R. Pomraning***, *Siwei Wei, Sue A Karagiosis, Young-Mo Kim, Alice Dohnalkova, Mary S Lipton, Galya Orr, Thomas O Metz, Scott E Baker.* Pacific Northwest National Lab, Richland, WA.
Yarrowia lipolytica is an oleaginous ascomycete yeast that accumulates large amounts of lipids. Despite a growing scientific literature focused on lipid production by *Y. lipolytica*, there remain significant knowledge gaps regarding the key biological processes involved. We are taking a multidisciplinary approach to identifying and characterizing the key pathways involved in *Y. lipolytica* lipid accumulation. A combination of -omic approaches, microscopy, and forward and reverse genetics is being applied to deepen our understanding of how this organism regulates and controls lipid production. We have analyzed lipid accumulation by *Y. lipolytica* growing in shake flasks and have developed a fluorescence based genetic screen for mutant strains with altered lipid accumulation.

434B. Influence of the nitrogen source on the production of volatile aroma compounds by non-conventional *Saccharomyces* species. ***Jiri Stribny***, *Roberto Pérez-Torrado, Amparo Querol.* Food Biotechnology, Institute of Agrochemistry and Food Technology, Paterna (Valencia), Valencia, Spain.
Nitrogen is one of the most important yeast nutrient which significatnly influences fermentation. The use of different nitrogen sources to avoid its deficiency in the fermentation process has become a common practice in the wine industry. Another common practice in winemaking of the recent years is introducing of some non-conventional species from *Saccharomyces* genus with unusual abilities. The species *S. kudriavzevii* and *S. uvarum* have shown very interesting properties leading to higher production of certain aromatic compounds. Since ammonium and amino acids are commonly used as a nitrogen supplement, we aimed to determine how *S. kudriavzevii* and *S. uvarum* manage these nitrogen sources and how they affect the production of volatile aroma compounds. The results show differences especially in the total volatile compounds composition giving *S. uvarum* as a great producer of acetate esters while *S. kudriavzevii* dominates in the production of higher alcohols. Based on this information we decided to compare the homologous genes from *S. cerevisiae*, *S. kudriavzevii* and *S. uvarum* involved in the flavour compounds synthesis. The *in silico* analysis identified the most radical substitutions in *ARO10*, *ATF1* and *ATF2* genes. When expressed in winery strain of *S. cerevisiae*, the increased amount of several aromatic compounds was observed. We detected increase of the production of banana and fruity flavours, such as isoamyl alcohol, isobutanol and their esters. Enzymatic assay also revealed interesting differences among the Aro10 proteins where Aro10p from *S. kudriavzevii* shows no preference to measured substrates while for *S. cerevisiae* Aro10p phenylpyruvate is the preffered substrate. Our results and the fact of close phylogenetic relationship among the three species suggest that the modification of overall wine aroma could be reached only by substitution of a few nucleotides.

435C. Evaluating Common Humoral Responses Against Fungal Infections With Yeast Protein Microarrays. ***Paulo Coelho***[1,2], *Hogune Im*[3], *Karl Clemons*[1,2], *Michael Snyder*[3], *David Stevens*[1,2]. 1) Dept of Medicine, Stanford University, Palo Alto, CA; 2) Calif. Inst. Med. Res., San Jose, CA; 3) Dept of Genetics, Stanford University, Palo Alto, CA.
In this study we profiled the global immunoglobulin response against fungal infection by using yeast protein microarrays. Groups of CD1 mice were infected systemically with human fungal pathogens (*Coccidioides posadasii*, *Candida albicans* or *Paracoccidioides brasiliensis*) or inoculated with PBS as a control. Another group was inoculated with heat-killed yeast (HKY) of *Saccharomyces cerevisiae*. After 30 days, sera from the individual groups were collected, pooled and used to probe *S. cerevisiae* protein microarrays containing 4,800 full-length glutathione S-transferase (GST)-fusion proteins. Anti-mouse IgG conjugated with alexafluor 555 and anti-GST antibody conjugated with alexafluor 647 was used to detected antibody-antigen interactions and presence of GST-fusion proteins, respectively. Serum from infection with *C. albicans* reacted with 121 proteins; *C. posadasii*, 81; *P. brasiliensis*, 67; and after HKY, 63 proteins on the yeast protein

microarray. We identified a set of 16 antigenic proteins that were cross-reactive among the three fungal pathogens. These include retrotransposon capsid proteins, heat shock proteins, and mitochondrial proteins. Some of the same proteins were identified in our previous study of fungi by mass spectrometry (Ann N Y Acad Sci. 1273:44-51). We observed 18 unique antigens between *P. brasiliensis* and *C. posadasii*, two phylogenetically closely related organisms; 4 unique proteins were found with other pairs. The results obtained give a comprehensive view of the immunological responses to fungal infections at the proteomic level. They also offer insight into immunoreactive protein commonality amongst several fungal pathogens, and provide a basis for a panfungal vaccine.

436A. The caveolin-binding motif of the pathogen-related yeast protein Pry1, a member of the CAP protein superfamily, is required for in vivo export of cholesteryl acetate. ***Rabih Darwiche***[1], *Vineet Choudhary*[1], *David Gfeller*[2], *Olivier Michielin*[2], *Vincent Zoet*[2], *Roger Schneiter*[1]. 1) Division of Biochemistry, Department of Biology, University of Fribourg , 1700 Fribourg, Switzerland; 2) Molecular Modeling, Swiss Institute of Bioinformatics , Quartier Sorge, Batiment Genopode, 1015 Lausanne, Switzerland.
Proteins belonging to the CAP superfamily (**c**ysteine-rich secretory proteins, **a**ntigen 5, and **p**athogenesis related 1 proteins) are found in all kingdoms of life and have been implicated in a variety of physiological processes, including immune defense, venom toxicity and sperm maturation. The molecular mode of action of CAP superfamily members, however, is poorly understood. The *S. cerevisiae* genome contains three members of this protein superfamily, Pry1,2,3 (**p**athogen **r**elated in **y**east). We have recently shown that Pry function is required for the secretion of cholesteryl acetate in yeast and that these proteins bind cholesterol and cholesteryl acetate *in vitro*, suggesting that members of this superfamily may generally act to bind sterols or related small hydrophobic components. Here we analyzed the mode of sterol binding by yeast Pry1. Computational modeling indicates that ligand binding could occur through displacement of a relatively poorly conserved flexible loop, which in some CAP family members displays homology to the caveolin-binding motif. Point mutations within this motif abrogated export of cholesterol acetate but did not affect binding of cholesterol. Mutations of residues located outside the caveolin-binding motif, or mutations in highly conserved putative catalytic residues had no effect on export of cholesteryl acetate or on lipid binding. These results indicate that the caveolin-binding motif of Pry1, and possibly of other CAP family members, is crucial for selective lipid binding and that lipid binding may occur through displacement of the loop containing this motif.

437B. Phosphoproteome analysis of the DNA damage response during S phase in Saccharomyces cerevisiae. ***Dongqing Huang***, *Brian Piening, Corey Weinert, Amanda Paulovich.* Clinical Division, Fred Hutchinson Cancer Research, Seattle, WA.
In response to replication stress, an extensive phospho-signaling cascade is activated and required for coordination of DNA repair and replication of damaged template (intra-S phase checkpoint). How phospho-signaling coordinates DNA replication stress response is largely unknown. We employed late generation of liquid chromatography tandem mass spectrometry technique (LC-MS/MS) to generate high-coverage and quantitative phospho-proteomic profiles of yeast exposed to the DNA alkylating agent methyl methanesulfonate (MMS). Our global scan identified 29,083 unique peptides representing the products of 4,503 unique genes. Our phospho scan identified 19,872 unique phosphopeptides representing the products 3,332 unique genes, in which 561 phosphopeptides (mapping to 457 unique proteins) were identified whose abundance changed 2-fold in response to MMS. The screen enabled detection of nearly all of the major proteins known to be present at the replication fork and/or required for the DNA damage response, including many novel MMS-induced phosphorylations. In order to identify phospho-sites that carry physiological importance, we engineered single or clustered mutations on a subset of proteins previously known to be involved in DNA replication, repair or lesion bypass. Interestingly, elimination of MMS-induced phosphorylation resulted in significant increases in MMS sensitivity or mutation rates in most of the mutants, indicating that induced phosphorylation is required for maintaining genome stability during replication stress. These results provide useful information for a better understanding of the regulation of replication stress by phosphorylation.

438C. Functional profiling of the ubiquitin-proteasome system of protein degradation. ***Anton Khmelinskii***[1], *Bernd Fischer*[2], *Joseph D Barry*[2], *Matthias Meurer*[1], *Daniel Kirrmaier*[1], *Michael Costanzo*[3], *Charles Boone*[3], *Wolfgang Huber*[2], *Michael Knop*[1]. 1) Center for Molecular Biology of the University of Heidelberg (ZMBH), DKFZ-ZMBH Alliance, Heidelberg, Germany; 2) European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany; 3) Banting and Best Department of Medical Research and Department of Molecular Genetics, The Donnelly Centre, University of Toronto, Toronto, Ontario, Canada.
Selective protein degradation contributes to cellular homeostasis through removal of unnecessary or damaged proteins. The ubiquitin-proteasome system (UPS) plays a key role in selective protein degradation, whereby a cascade of E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin-protein ligase enzymes marks proteins with polyubiquitin chains for degradation by the proteasome. Deubiquitinating enzymes (DUBs), which remove ubiquitin marks from target proteins and replenish the pool of free ubiquitin, are involved at various stages of the targeting and degradation processes.

Despite the central role of the UPS in protein degradation, many UPS components are poorly characterized, various E3 ligases have no known substrates and the functions of DUBs are not well understood.
To help bridge these gaps, we generated a genome-wide library tailored for in vivo analysis of proteome dynamics in the budding yeast *Saccharomyces cerevisiae*. We describe the construction of this library in which each of ~4000 strains expresses a different protein endogenously tagged with a tandem fluorescent protein timer (tFT). The tFT is composed of two fluorescent proteins with distinct kinetics of fluorophore maturation and reports on the abundance and degradation kinetics of the tagged proteins.
We applied this resource to systematically analyze the role of different UPS components in proteome turnover. Using synthetic genetic array technology followed by high-throughput whole colony fluorescence imaging, the abundance and stability of each fusion were measured in strains carrying mutations in key UPS components, including 11 E2 ubiquitin-conjugating enzymes, ~100 E3 ubiquitin-protein ligases and accessory subunits, and 20 DUBs. Analysis of this dataset provides insights into the organization of the UPS in terms of specificity and interplay of different enzymes, defines functions for several poorly characterized UPS components and identifies novel potential substrates of different E3 ubiquitin-protein ligases.

439A. Stable-seq: High-throughput Analysis of in vivo Protein Stability. ***Ikjin Kim***[1], *Christina Miller*[1,2], *Stanley Fields*[1,2,3]. 1) Department of Genome Sciences, University of Washington, Seattle, WA, USA; 2) Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA; 3) Department of Medicine, University of Washington, Seattle, WA, USA.
Current methods for measuring in vivo protein stability, including large-scale approaches, are limited in their throughput or in their ability to discriminate among small differences in stability. We developed a method, Stable-seq, that uses a simple genetic selection combined with high-throughput DNA sequencing to assess the in vivo stability of proteins. To this end, proteins or peptides are fused to a metabolic enzyme, the yeast Leu2 protein. These fusion proteins accumulate to different levels based on their stability and lead to different growth rates when the yeast are grown in the absence of leucine. High-throughput sequencing of the input population of proteins and the population after leucine selection allows the stability of either many variants of a protein or many different proteins to be scored in parallel. By applying the Stable-seq method to variants of the protein degradation signal Deg1 from the yeast Mat2 protein, we generated a high-resolution map that reveals the effect of ~30,000 mutations on protein stability. We are also applying Stable-seq in an effort to define yeast substrate-E3 ubiquitin ligase pairs. Of more than 120 yeast E3 enzymes, only a few have defined substrates, even though more than 2,000 yeast proteins are ubiquitinated. If the leucine selection is carried out in a yeast strain deleted for an E3 enzyme necessary for degradation of a Leu2 fusion, then cells bearing this fusion should grow faster than when the fusion is present in the wild type yeast background. With the Deg1-Leu2 fusion, a strain deleted for the DOA10 gene, encoding the relevant E3 enzyme, resulted in the greatest increase in stability. To delineate the substrate specificity of E3 enzymes, we generated a library of random 20 NNK codons fused to the LEU2 gene. By assaying the library in a few E3 deletion strains, we have identified peptides whose stability is increased compared to the wild type strain. We are also generating a library of yeast ORFs fused to LEU2, which will be used to measure the stability of yeast proteins in wild type and E3 deletion backgrounds.

440B. Proteomic analysis and metabolic exploration of *Yarrowia lipolytica* under different culture conditions. ***J. Shi***, *W. Chen*. Nanyang Technological University, Singapore.
Yarrowia lipolytica is an oleaginous yeast which has been proved to be capable of producing significant quantities of citric acid. It is also widely used for single-cell oil production. In our study, when glucose was utilized as carbon source to culture *Y. lipolytica*, citric acid production was induced under flask nitrogen-limited cultures, whereas the production of intracellular lipid was not favored. Maximum concentration of citric acid was up to 27 g/L (yield 0.45 g/g glucose), while total lipid amount was merely around 10% of biomass. Interestingly, when cells were grown on oleic acid, cellular lipid production was favored and highest concentration was detected to be up to 3.3 g/L (yield 0.33 g/g oleic acid). However, low quantities of citric acid was detected with amount only up to 1.1 g/L. Comparative proteomics analysis of *Y. lipolytica* was performed to investigate the effect of glucose and oleic acid on production of citric acid and reserve lipid under intracellular nitrogen limitation condition. Two dimensional LC-MS/MS approach has been applied for protein profiling together with isobaric tag for relative and absolute quantitation (iTRAQ) labelling method. 195 proteins were identified within cells collected during citric acid accumulation stage and lipid accumulation stage, when the strain was cultured with glucose and oleic acid as carbon sources, respectively. Significantly up-regulation or down-regulation of proteins were experienced among comparison. Essential proteins correlated to the synthesis and regulation of citric acid and lipid were detected. Our approach contributes to better understanding of *Y. lipolytica* metabolism from proteomic level and could further contribute to genetic engineering for higher yield of valuable metabolites production.

441C. Identifying global changes in protein acetylation following heat shock. ***Rebecca E. Sides***, *Jeffrey A. Lewis.* Biological Sciences, University of Arkansas, Fayetteville, AR.
All organisms face diverse environmental stresses throughout their lifetime. Microbes, including budding yeast, face the

added disadvantage of being unable to flee from stressful environments. Thus, a battery of rapid stress defense mechanisms is critical for survival. One potential regulatory mechanism for rapid adaptation to stress is post-translational protein acetylation. Global acetylation studies in yeast have found that over a sixth of the proteome contains lysine acetylation under standard conditions, with an enrichment for proteins involved in metabolism, translation, and stress defense. It is also well established that for specific proteins, acetylation states vary depending upon environment. This suggests that protein acetylation is a common regulatory feature, and yet we have limited knowledge of the function of protein acetylation for the majority of proteins. To gain insight into the potential functions and better understand the role of protein acetylation globally, we analyzed the acetylome using 2D gel electrophoresis and SILAC-based proteomics for both unstressed cells and cells responding to mild heat shock. Our data clearly demonstrates acetylome remodeling during acute heat shock. We are currently using molecular genetics and biochemistry to study the effects of protein acetylation on representative stress defense proteins. Ultimately, this approach will provide new insight into the role of global acetylation during the response to acute stress.

442A. Identification of long-lived proteins in cells undergoing repeated asymmetric divisions. ***NH Thayer***[1,2,3], *C Leverich*[1,3], *E Marsh*[1], *M Fitzgibbon*[1], *ZW Nelson*[1], *KA Henderson*[1], *J Hsu*[1], *DE Gottschling*[1]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 3) These authors contributed equally to this work.
Long-lived proteins are implicated in the age-associated decline of biological processes. Previous studies of post-mitotic mammalian cells have identified a number of long-lived proteins, some of which may play a role in various aging processes, however these systems have been unable to assay mitotically-active cells, e.g. stem cells. It has been proposed that some factor or combination of factors are retained in asymmetrically dividing cells and this contributes to the cellular aging process. The budding yeast, *Saccharomyces cerevisiae*, provides an opportunity to identify such factors, because *i*) dividing mother cells experience a finite replicative lifespan and *ii*) large numbers of aged cells can be obtained. We sought to identify any proteins that are long-lived and asymmetrically retained in aging mother cells, possibly leading to various aging phenotypes and ultimately to senescence. By combining our previously described Mother Enrichment Program, a pulse-chase heavy-isotope labeling strategy, and mass spectrometry, we have identified ~130 proteins that appear to be both long lived and asymmetrically retained in the mother cell. We refer to these proteins as Long-lived Asymmetrically Retained Proteins (LARPs). Using the RITE-tag system (Verzijlbergen et al. 2010), we continued to classify these proteins into several groups. Two types of LARPs were discovered, full-length and fragmented. The full-length category contains different classes of protein: plasma membrane proteins (e.g. Mrh1p, Pma1p, and Sur7p) which show near 100% asymmetry where none of the mothers protein is transferred to the daughter; cytoplasmic proteins (e.g. Hsp26p and Thr1p) which form foci are extremely long-lived and have a propensity to stay in the mother cell; and cell wall proteins (Bgl2, Exg1, Pho5, Pho11). The fragmented group consisted primarily of proteins involved in translation and protein folding, or glycolytic enzymes. These fragmented LARPs appear to be partially degraded products retained in the mother cell. We propose that LARPs, both full-length and fragmented, contribute to the aging of cells that undergo repeated asymmetric cell divisions.

443B. A systematic investigation of small metabolite interactions with regulatory proteins in yeast. ***G.X. Yang***, *X. Li, M. Bruno, M. Snyder*. Department of Genetics, Stanford University, Stanford, CA.
Natural small metabolites comprise the majority of cellular molecules and participate in a wide variety of biochemical and regulatory processes. However, a global analysis of their regulation of cellular process through direct binding and regulation of protein activity is lacking. Our lab has begun to systematically investigate *in vivo* protein-metabolite interactions. In an initial study, we had widespread interactions of hydrophobic metabolites with different groups of proteins found by mass spectrometry (Li *et al*. Cell, 2010). We are now extending this untargeted and systematic approach to investigate the interaction of both hydrophilic and hydrophobic metabolites with every protein produced by S. cerevisiae. In particular, we are analyzing 200 yeast proteins (3% of the whole proteome) that are known to regulate enzyme activities based on the GO annotation. We have tailored the methods of metabolite extraction and screening to be suitable for analyzing hydrophilic molecules. We have found that many binding interactions are in accordance with the reported protein functions. For instance, Bcy1, a regulatory subunit of the cyclic AMP-dependent protein kinase, shows significant binding to cAMP and adenine. We also found that many proteins (7% of investigated) bind to ergosterol, which supports the hypothesis given in Li *el al* s paper that ergosterol is a global regulator for yeast proteins. Intriguingly, we also discovered numerous novel interactions. For example, Vhs3, a regulatory subunit of protein phosphatase 1 Ppz1p and a homolog of an FMN-binding protein, binds to an endogenous product of riboflavin, which is the central component of FMN. Characterization of these interactions is in progress. These preliminary results reveal extensive metabolite-protein interactions in yeast by confirming and discovering both hydrophobic and hydrophilic protein-bound metabolites. A systematic investigation of such interactions provides new insights about the regulation of protein functions. Such knowledge might have important implications in promoting human health by correcting faulty protein functions. It can also facilitate the design of drugs that are metabolite analogs while preventing potential side effects.

444C. Measuring stress response in Saccharomyces cervisiae using Isotopic Ratio Outlier Analysis (IROA) for metabolome-wide quantitation. ***Felice A de Jong****, Chris Beecher.* IROA Technologies LLC, Ann Arbor MI.
The response to a stressor ultimately represents a disruption of homeostasis, which may be viewed either at the level of the epigenome, the transcriptome, or the metabolome. In most cases the metabolome is the earliest responder. The IROA approach was used to determine the global biochemical response of the model system Saccharomyces cerevisiae S288C to three well characterized toxins, Ketoconazole, Terbinafine, and 5-flourocytosine. S. cerevisiae was maintained in media in which all of the carbon was isotopically defined (95% or 5% 13C) until fully transformed to the isotopic balance of the media prior to exposure to toxin or vehicle control for a 24 hour period. Control and experimental samples were pooled to mitigate commonly encountered sources of variance, including sample-to-sample, prep-related, and ion suppression. Pooled samples were extracted, then separated and analyzed using LC-MS. The approach enables the removal of false data such as noise or artifactual peaks, identified by the absence of isotopic signature. IROA software performs a scan-by-scan analysis of the LC-MS dataset and identifies IROA peaks based on their extended isotopic envelopes. Peaks of biological origin are perfectly paired; each IROA envelope is half control and half experimental. The ratio of these halves quantitates the stressor response relative to the control. As an example, the IROA envelope for the metabolite glutathione (C10H17N3O6S) has two halves separated by a 10 carbon mass difference. The sum of the base peaks and their associated isotopic peaks are used to calculate their respective areas accurately and their ratio is evaluated. In this study, 499 IROA peaks were identified, quantitated and classified using both supervised and unsupervised methods. Principal Component Analysis and Self-organizing Maps cleanly separated the control and each of the three toxin sample types and Random Forest yielded a confusion table with zero error. The metabolic response profiles for each toxin were unique and important discriminating compounds included N-acetyl-arginine, nicotinamide, succinic anhydride, glutathione, leucine and the peptide cys-gly. The IROA protocol coupled with multiple classification techniques provided insight to common features and variations in yeast responses profiles for each toxin studied.

445A. 50:50 and IpO: Two new PCR-based methods for marker-free, seamless genome modification in yeast. ***Joe Horecka****, Angela Chu, Davis Ronald.* Biochemistry, Stanford University, Stanford, CA.
The ability to modify the yeast genome with relative ease has contributed to the organism being a model eukaryote for decades. We developed two PCR-based methods that streamline marker-free, seamless genome modification. Both are based on classical two-step gene replacement. The 50:50 method is amenable to the facile creation of deletions and short insertions or substitutions. It requires only two primers, one PCR with a URA3 cassette, and a single transformation. The second method can be used to insert DNA segments. The design of a new vector, IpO, reduces PCR template plasmid construction to the cloning a single copy of the DNA segment between the vectors split-URA3 fragments. Two PCRs are performed that capture the DNA segment and one or the other URA3 fragment. When the PCR products are co-transformed into yeast, recombination between the overlapping URA3 fragments restores URA3 and transposes the cloned DNA segment inside out, creating a repeat-URA3-repeat cassette. Sequences designed into the PCR primers target integration of the cassette into the genome. Subsequent selection with 5-fluoro-orotic acid yields strains that have popped out URA3 via recombination between the DNA repeats, with the result being the precise insertion of the DNA segment minus the selectable marker. An additional advantage of the IpO method is that it eliminates PCR artifacts that can plague the current methods repeat-containing templates.

446B. Multiplex, marker-less genetic engineering in *S. cerevisiae*. ***Andrew Horwitz****, Max Schubert, Jack Newman.* Amyris, Emeryville, CA.
As the sophistication of metabolic engineering increases, so do the number of chromosomal loci that must be engineered in the host organism. Performing these genetic transformations in a serial manner represents a rate limiting step. To address this barrier and accelerate the design-build-test cycle, we have developed a robust protocol for parallel genome engineering in *S. cerevisiae*. As a proof of principle, we built a platform strain with rare restriction sites integrated at three loci, and attempted to achieve triple, marker-less integrations by co-transforming a plasmid encoding the restriction endonuclease. We observed that multi-locus, marker-less integrations are improbable using standard techniques, but become nearly inevitable with expression of a meganucleases or designer endonuclease targeting the integration loci. By integrating nuclease recognition sites near copies of genes encoding the terminal synthase in a sesquiterpene pathway, we were able to swap up to 8 synthases simultaneously, effectively compressing 1.5 months of serial engineering into 1 week. Swapping the original synthases for evolved versions led to stepwise increases in titer, and swapping alternate synthases changed the product profile in a single step. The observed efficiency of swapping presumably derives from both an increase in homologous recombination at targeted sites as well as a strong genomic selection against cells that fail to recombine the donor DNA. This result demonstrates that inducing double strand breaks in the *S. cerevisiae* genome is sufficient to drive multiplex, marker-less integrations. We are currently developing methods for delivering multiple designer endonucleases simultaneously, the achievement of which will allow us to extend this technique to arbitrary native loci in *S. cerevisiae* and to alternative fungal hosts.

447C. Biosensors engineered from conditionally stabilized ligand-binding domains. ***Benjamin Jester***[1,2], *Christine Tinberg*[3], *Justin Feng*[4], *Dan Mandell*[5], *George Church*[5], *David Baker*[1,3], *Stanley Fields*[1,2,6]. 1) Howard Hughes Medical Institute, University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Department of Biochemistry, University of Washington, Seattle, WA; 4) Division of Medical Sciences, Harvard Medical School, Boston, MA; 5) Department of Genetics, Harvard Medical School, Boston, MA; 6) Department of Medicine, University of Washington, Seattle, WA.
Transcription factors (TFs) engineered to conditionally respond to the presence of specific small molecules would be useful tools for the detection of environmental contaminants, orthogonal gene induction, and metabolic engineering. Most protein-based biosensors measure analyte concentrations directly and exhibit narrow dynamic ranges. Beginning with a minimal scaffold comprised of a DNA-binding domain and transcriptional activation domain, we developed a sensor in which ligand-induced stabilization of the TF activates expression of a reporter gene. Incorporation of an unstable ligand-binding domain into the TF scaffold generates a destabilized protein that is quickly cleared from the cell and drives only a low level of reporter gene expression. Addition of the cognate ligand stabilizes the ligand-binding domain and increases in vivo levels of the TF, thus coupling transcriptional activation to the level of the small molecule. Relative to previous methods that have used conditional degrons to regulate protein activity by direct fusion, a TF-based system should be more sensitive, have a wider dynamic range, and be more modular, at the expense of a rapid response time. As a proof of principle, we fused an engineered digoxigenin-binding protein, DIG10.3, to the DNA-binding domain of Gal4 and a VP16 activation domain, and demonstrated the function of this sensor in S. cerevisiae. Though the initial sensor was capable of inducing reporter expression in the presence of digoxigenin, FACS analysis of a library of DIG10.3 variants allowed us to identify conditionally stable mutants that enhanced induction by several-fold. Reducing the potency of the activation domain yielded further improvements in sensor function. To enhance the sensitivity of the sensor, we deleted several ABC transporters from the strain in order to restrict efflux and increase intracellular levels of exogenously added ligand. This approach should be generally applicable to small molecules for which a binding protein either exists or can be designed.

448A. Acetylation of carotenoids in *Yarrowia lipolytica* improves cell viability and titers. ***Lisa Laprade***, *Maria Mayorga, Chris Farrell, Peter Houston, Dan Grenfell-Lee, Joshua Trueheart.* DSM Microbia, Lexington, MA.
The large lipid bodies generated by the oleaginous yeast *Yarrowia lipolytica* are an excellent storage organelle for a large class of hydrophobic carotenoids. In nature *Yarrowia* does not make carotenoids. We have successfully engineered *Yarrowia* by introducing heterologous biosynthetic activities, enhancing the native isoprenoid pathway, and utilizing classical breeding to produce increasing titers of -carotene, canthaxanthin, and lycopene in lab and large scale fermentors. In contrast, the hydroxylation of -carotene and canthaxanthin, resulting in zeaxanthin and astaxanthin, respectively, dramatically degrades the cells ability to tolerate high concentrations of carotenoid: large crystals precipitate from the lipid bodies; cells rapidly lose viability; and carotenoid production plateaus. The introduction of a promiscuous acetyl transferase, *ATF1*, from various *Saccharomyces* species results in greater than 90% esterification of zeaxanthin and astaxanthin, a marked slowing of cell death, and a significant increase in carotenoid titers. Current titers represent the first commercially viable carotenoid levels achieved through genetic engineering.

449B. Phenomic Analysis of TOR Signaling and dNTP Metabolism. ***Sean M. Santos***, *Chandler Stisher, Darryl Outlaw, Jingyu Guo, John L. Hartman, IV.* Department of Genetics, University of Alabama at Birmingham, Birmingham, AL.
We are developing a phenomics workflow for modeling human disease-related processes, which includes quantitative high throughput cell array phenotyping (Q-HTCP), recursive expectation-maximization clustering (REMc), and Gene Ontology (GO) analysis. Q-HTCP is a robotic method to collect growth curve data and is applied to the entire collection of S. cerevisiae deletion strains, yielding cell proliferation parameters (CPPs) for measuring gene interaction, which are in turn subjected to REMc followed by hierarchical clustering. The workflow provides automated generation of clusters with heat maps and biological annotation by GO Term Finder (GTF). In this study, we are comparing the gene interaction landscapes for target of rapamycin (TOR) signaling and dNTP metabolism. Gene-drug interaction screens were performed for rapamycin, temsirolimus, and hydroxyurea on fermentable and non-fermentable media. In addition, the SGA method was used to introduce tetracycline-regulated alleles of GLY1, RNR1, THR1, TOR1, and TOR2 into the knockout collection. Q-HTCP provided CPPs, which were used to calculate gene interaction values, and the resulting matrix was passed through the clustering workflow. Heat maps revealed distinct patterns for each cluster and GTF reported enrichment in cellular functions shared by genes within each cluster. Gene interaction data for the 11 perturbations outlined above gave rise to 177 clusters in four rounds of clustering, associated with enrichment (p0.05) in 681 GO terms, including 392 relatively specific GO terms (50 genes annotated to the term). We found this workflow useful for organizing phenomic data into biological modules according to patterns of functional gene interaction. However, while highlighting cellular processes related by genetic interaction, the overall result is not yet easily interpreted with respect to biology underlying the various cellular processes and array of perturbations tested. Thus, additional strategies are being pursued to understand the myriad of phenotypic effects resulting from all the perturbations tested. An automated strategy of this type would facilitate successful application of the unique capabilities of yeast phenomics in models of human disease aimed at

understanding genetic and phenotypic complexity. Other ongoing studies to identify genetic and biological relationships between TOR signaling and dNTP metabolism include directly measuring dNTP pools and other metabolites in the context of informative gene interaction. The ultimate goal is to understand whether loss of the normal coordination between these aspects of cellular metabolism provide a vulnerability for attack by targeted therapeutics in cancer.

450C. An automated system for time-lapse imaging of microbial biofilms. ***Adrian Scott****, Aimée M. Dudley.* Pacific Northwest Diabetes Research Institute, Seattle, WA.
Biofilm formation is an important process in many microorganisms, including opportunistic human pathogens such as Pseudomonas aeruginosa and Candida albicans. Many strains of Saccharomyces cerevisiae can form biofilms with colonies that have shapes that are both distinct and genetically determined. These colonies change dramatically over both short and long time scales, from localized "pops", which take place over less than 5 minutes, to large-scale structures that develop in the colony over the course of several days. In order to capture detailed measurements of morphology dynamics, colonies must be photographed frequently for days at a time under constant temperature and colony spacing conditions. We have constructed an automated imaging platform that acquires high resolution (up to 750 pixels/mm) color photographs of evenly spaced single colonies (up to 48 colonies per plate). The system is composed of 3 cameras, each mounted on a 3-axis computer-controlled frame. Each DSLR camera is outfitted with a variable-magnification macro lens, allowing for magnifications from 1x to 5x. The cameras can be moved on each axis via stepper motors controlled by a Computer Numerically Controlled (CNC) board. Above each camera is a clear acrylic sheet with space to hold up to 12 rectangular agar plates, permitting the imaging of over 1,700 single colonies in a single experiment. Colonies are evenly spaced on the agar plates by using a cell sorter to deposit single cells in a grid pattern onto each plate. All three cameras are mounted inside a large incubator to maintain constant temperature. The CNC boards and cameras are controlled by a laptop, which moves each camera to a colony, acquires an image, and then moves to the next colony. Once every colony has been imaged, the camera returns to the first colony and repeats the process. This system provides continuous photographing of individual colonies during the entirety of their development. While we have applied this system to the study of colony morphology in S. cerevisiae, it is compatible with any colony forming microorganisms that can be grown on agar plates.

451A. Development of ODELAY, a scalable, automated, multiparameter growth rate analysis platform, and application to a yeast model of Parkinson's disease. *David J. Dilworth[1], Alexander V. Ratushny[1,2], Thurston E. Herricks[1],* ***Jennifer J. Smith****[1], Song Li[1], John D. Aitchison[1,2].* 1) Institute for Systems Biology, Seattle, WA; 2) Seattle Biomedical Research Institute, Seattle, WA.
The majority of current systems genetics studies that employ growth as a metric of fitness do so at only a single time point and the absence of a temporal dimension, significantly limiting the sensitivity of these analyses. In order to study phenotypic effects at a higher resolution, we developed a novel approach to do live, single cell analyses of colony forming microorganisms. This approach, ODELAY (One-cell Doubling Evaluation by Living Arrays of Yeast), provides sensitive and quantitative high-density measurements of strain doubling times during exponential growth, lag times prior to entry into exponential growth, and population at carrying capacity derived from microcolonies expanding from single cells to up to thousands of cells grown on solid media. We are applying ODELAY for high content phenotyping of a yeast model of -synuclein toxicity to understand cellular processes involved in neurological disorders like Parkinsons disease, with focus on ER to Golgi vesicle trafficking, and peroxisome biogenesis and function. Clustering analysis of multiparameter ODELAY data yielded sensitive and high-resolution strain classification, revealing biological insights not previously obtained from single time-point growth studies.

452B. Genome Wide Manipulation of Transcription in Saccharomyces cerevisiae using CRISPR-Cas9 Transcription Factors. ***Justin D Smith****[1,2], Ulrich Schlecht[2], Sundari Suresh[2], Ron W Davis[2], Leopold Parts[3], Robert P St. Onge[2].* 1) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 2) Stanford Genome Technology Center, Stanford University, Palo Alto, CA; 3) Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany.
Bacterial type II CRISPR-Cas9 systems have been widely adapted for RNA-guided genome editing and transcription regulation in eukaryotic cells. We have optimized different activator and repressor fusions, and assessed the effects of guide position, to optimize Cas9-mediated transcriptional regulation in yeast. Using these data we developed a computational method that uses published data to select guides that activate or repress genes with superior potency. As a proof of concept we activated and repressed several known drug targets, investigated the effects of transcriptional changes on the protein-protein interaction network, and investigated the effects of guides targeting bidirectional promoters. We also developed an approach to express multiple guide RNAs from a single transcript, thereby allowing us to efficiently target multiple genes within the same yeast cell. We are currently applying these methods to answer a variety of questions on a genome-wide scale using competitive assays involving pools of strains expressing individual guide RNAs.

453C. Seeking a metabolic understanding of chronological aging. ***Haley Albright****, Crystal Maharrey, Daniel Smith, John L. Hartman IV.* Department of Genetics, University of Alabama at Birmingham, Birmingham, AL.
The chronological lifespan (CLS) model is used to understand cellular aging in post-mitotic cells, which is measured by a decline in the percentage of colony forming units in stationary culture over time. We performed a phenomic screen for CLS in the *S. cerevisiae* deletion library, which revealed several metabolism-related genes. As reported by the Kaeberlein laboratory and confirmed by others, cells grown in buffered media exhibit a longer CLS than cells grown in unbuffered media. We investigated this buffering effect in the context of developing an assay to measure intracellular TCA metabolite concentrations by LC-MS. TCA metabolite pools, extracellular pH, and colony forming unit measures were collected for several weeks, comparing buffered and unbuffered media. Pool levels varied in metabolite-specific patterns between the two media conditions, and taken together, suggested four time windows between which metabolic transitions occurred. The time windows were logarithmic growth (day 1), diauxic shift (day 2-3), establishment of stationary phase (~3-4 weeks), and quiescence. During logarithmic phase, pool levels were comparable between the two media for most metabolites. However, during the diauxic shift, metabolite pool levels were more variable, settling out at higher levels in unbuffered media for several of the metabolites in early stationary phase, only to eventually again become equivalent, and sometimes higher, in buffered cultures as cells entered quiescence. Succinate exhibited a distinct profile with pools rising much higher in buffered media relative to unbuffered media during the establishment of stationary phase. We also noted from the CLS screen that deletion of genes functioning as part of the succinate dehydrogenase (SDH) complex altered CLS. Knockout of genes encoding other TCA cycle enzymes also influenced the CLS phenotype, but this was not necessarily reflected by changes in cognate metabolites when comparing pool profiles in buffered and unbuffered media. Taken together, the results suggest that further genetic and metabolic studies of TCA cycle activity and electron transport will yield insight into biochemical mechanisms for chronological aging. Ongoing and future studies include non-targeted (unbiased) metabolomics and assessment of metabolic fluxes, additional genome-wide CLS screens with various media conditions, and utilization of assays for mitochondrial function employing fluorescent dyes and flow cytometry.

454A. Systems biology guided engineering of reduced ethanol production in industrial wine yeast. ***Anthony R. Borneman****, Cristian Varela, Simon Schmidt, Paul J. Chambers, The Australian Wine Yeast Systems Biology Consortium.* Wine Biosciences, The Australia Wine Research Institute, South Australia, Australia.
The ability to interrogate genome-wide biological datasets as part of a systems biology framework is poised to revolutionize the development of industrial microorganisms such as the yeast *S. cerevisiae*. Over recent years, laboratory strains of *S. cerevisiae* have been applied at the cutting edge of Systems Biology research. However, relative to laboratory strains, industrial *S. cerevisiae* strains, such as those used in baking, brewing, winemaking and biofuel production, display very distinct phenotypes, such as increased stress tolerance and the production of key secondary metabolites that are critical for industrial applications. Given the intellectual and economic benefits that fundamental understanding of industrial yeasts will provide, we have undertaken a collaborative systems biology investigation of industrial wine yeast fermentation, analysing comparative genomic, transcriptomic (RNAseq), proteomic (2D-gels and iTRAQ) and metabolomic (targeted and non-targeted metabolomic profiling) data under model winemaking conditions. Furthermore, we have successfully applied these systems-level insights into fermentative metabolism to rationally engineer genetic alterations to counteract serious metabolic side-effects that can result from engineering yeast metabolism for the production of low-ethanol wine.

455B. Instant killing of yeast for protein studies during continuous culture. ***Sara S. Dick****, Khyla Rose Alorro, Sean McNabney.* Biology, Valparaiso University, Valparaiso, IN.
Saccharomyces cerevisiae is a well-used model system and is ideal for studying biological phenomena. Biological rhythms have not been well-studied thus far in yeast, although much has been found in other organisms. Yeast have the potential to be a useful model system for biological rhythms in addition to their utility in other systems. A continuous culture can detect rhythmic oscillations in several characteristics of the culture, including pH changes and levels of dissolved oxygen. But in order to identify genes and proteins that oscillate in activity or amount in a yeast culture over time, it is necessary to be able to take hourly or sub-hourly samples from a continuous culture. It is also necessary to fix the cells in the condition they were in at the time they were collected. Here we describe a method for automated sampling from a continuous culture in which the cells are killed as they are collected at room temperature, using no special equipment or conditions, allowing observation of gene expression and protein level or activity. Although this technique was designed to study biological rhythms in yeast, it has applications in many other experimental designs.

456C. Uncovering the biophysical impact of sequence variation. ***Kathryn M Hart****, Jasper Rine.* University of California Berkeley, Berkeley, CA.
Deciphering molecular mechanisms by which sequence variation leads to phenotypic variation is central to research in many areas of biology, ranging from evolution to human disease. In some cases, the effects of missense mutations on protein structure and function can be used to rationalize complex behaviors, but often the mechanisms connecting

molecular phenotype and organismal phenotype prove elusive. While most missense mutations are neutral from the perspective of organismal fitness in laboratory settings, nearly all will affect a proteins biophysical and energetic characteristics. To investigate the impact of a proteins biophysical traits on organismal fitness, we use dihydrofolate reductase (DHFR) as a model protein and measure how cells respond to changes in DHFRs thermodynamic and kinetic stabilities. Multidimensional phenotypes of DHFR stability variants are evaluated by synthetic genetic interactions with components of the proteostasis machinery in S. cerevisiae. By focusing on well-characterized variants of DHFR, we will connect biophysical understanding of sequence variation with its genetic impact. Defining the relationship between protein energetics and genetic interactions will also facilitate the characterization of novel variants based on their genetic interactions. Finally, the data acquired here will inform predictions of the functional effects of missense mutations for other proteins based on changes in stability rather than more traditional methods based on sequence conservation. Important features like global stability and dynamics are not easily mapped to one or even a handful of residues; rather, they are emergent properties that reflect complex interactions between all positions in a sequence. These properties provide a more comprehensive description of a protein than simply the conservation of a residue at a particular position. Harnessing the awesome power of yeast genetics to inform physical and energetic models of proteins will lead to mechanistic understanding of how mutations contribute to disease and provide the foundation for more sophisticated prediction methods.

457A. Reverse Two Hybrid, a systematic approach for identifying genes and pathways that regulate a specific protein-protein interaction. ***Ifat Lev***, *Marina Volpe, Shay Bev Aroya.* Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel.
Protein-protein interactions (PPIs) are of central importance for many areas of biological research. Several complementary high-throughput technologies have been developed to study PPIs. The wealth of information that emerged from these technologies led to the first maps of the protein interactomes of several model organisms. Many changes can occur in protein complexes as a result of genetic and biochemical perturbations, however in the absence of a suitable assay, such changes are difficult to identify, and thus have been poorly characterized. Recently we developed a high-throughput genetic approach (termed reverse PCA) that allows the identification of genes whose products are required for the physical interaction between two given proteins (PLOS Genetics. 2013) . Here we describe a complementary approach termed reverse Two Hybrid. In this assay we start with a yeast strain in which the interaction between two given proteins can be detected by the expression of a reporter gene (HIS3), in the context of the Yeast Two Hybrid assay (Y2H). Using the synthetic genetic array (SGA) technology, we can systematically screen mutant libraries of the yeast Saccharomyces cerevisiae to identify those mutations that disrupt the physical interaction of interest. We were able to successfully validate this novel approach by identifying mutants that dissociate the interaction between Dam1 and Ctf19, two subunits of the Kinetochore, a conserved complex where the spindle fibers attach during cell division to pull sister chromatids apart.. We believe that this method will facilitate the study of protein structure-function relationships, and may help in elucidating the mechanisms that regulate PPIs.

458B. Profiling of *Saccharomyces* yeast populations in two British Columbia vineyards and wineries. ***J. Martiniuk***, *V. Measday.* Wine Research Centre, University of British Columbia, Vancouver, BC, Canada.
Wine is created by the fermentation of sugars in red or white grape juice to ethanol, mainly by the wine yeast *Saccharomyces cerevisiae* (*S. cerevisiae*). Two winemaking methods are used: spontaneous fermentation, where yeast present on the grapes from the vineyard or on winery equipment carry out the fermentative process; and inoculated fermentation, where a commercially prepared, single strain *S. cerevisiae* inoculum is used. Spontaneously fermented wines are characterized by a diverse succession of yeast species and strains that vary by region. They can be more organoleptically complex and have more regional character than wines produced by inoculated fermentation, but are subject to greater risk of sluggish or stuck fermentation, and if present in the winery, commercial *S. cerevisiae* strain contamination. Using microsatellite analysis, we have identified the *Saccharomyces* species and strains present in the vineyards of two British Columbian (BC) wineries that use both inoculated and spontaneous fermentation methods. To determine the role of the winery environment in introducing different *Saccharomyces* species and commercial strains, we have compared vineyard yeast populations to their respective winery spontaneous fermentation populations. Our results indicate that in most winery spontaneous fermentations, *S. cerevisiae* strains indigenous to the vineyards were dominated by commercial strains introduced at the winery, and in some cases by commercial strains present in the vineyards themselves. In one case, *Saccharomyces bayanus* was identified in one winery vineyard and dominated its respective winery fermentation. To our knowledge, this is the first study in North America to examine vineyard *Saccharomyces* species and strain populations. More information about the *Saccharomyces* yeast populations in British Columbia wine regions will be highly valuable to the Canadian wine industry from a fermentation management perspective and from a resource perspective, as research in this area could reveal novel and unique species and/or strains that can be exploited to enhance a wines regional character.

459C. Staying current and modern: Overhauling an actively-used model organism database website. ***Kelley M. Paskov***, *Stacia R. Engel, Gail Binkley, J. Michael Cherry.* Department of Genetics, Stanford University, Stanford, CA.
The success of any website hinges on two things: (1) keeping content current and relevant, and (2) maintaining and extending the codebase. The Saccharomyces Genome Database (SGD; www.yeastgenome.org) is one of the most widely accessed model organism databases, averaging almost a million page views each month from almost a quarter-million unique visitors. In order to store new types of biological data and to leverage new web development tools and techniques, SGD is in the midst of a complete overhaul, both inside and out. We are restructuring pages, data transfer methods, and the underlying database schema, all while keeping the site live and actively curated. Continuing to maintain a site and its underlying database of constantly updated data, while developing a new one, presents a series of challenges. New pages must be integrated into the site to enhance usability and form a cohesive experience for the user. New data must be made available on a daily basis. Data and code must be checked for accuracy. We have addressed these challenges with several key tools, using ongoing cycles of rapid development to push new pages out in phases. The SQL toolkit, SQLAlchemy, allows us to more easily write data conversion code. Automated testing tools like Selenium help guarantee the reliability of new code. A variety of third-party tools (Pyramid, Google Charts, cytoscape.js, DataTables) allows quick design of new pages and data display. The ultimate goal is to redesign the SGD website to be faster and easier to maintain, and to provide a beTer user experience, without creating any downtime or curation lag for our users. Here we present an update on the ongoing implementation of the new SGD website and database that more effectively serves researchers access to experimental results.

460A. Underlying the phenotypic contribution of a single quantitative trait locus in yeast are effects of seven genes and their epistatic interactions. ***R. Shapira***, *T. Benbenishty, L. David.* ANIMAL SCIENCES, FACULTY OF AGRICULTURE, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, ISRAEL, REHOVOT, ISRAEL.
Quantitative traits are characterized by a continuous distribution of phenotypic values. Many human and animal diseases as well as production traits in crop plants and farm animals are quantitative traits, creating a long-standing interest in understanding their genetic basis. Contributing to phenotypic variation in such traits are many genes with variable effect sizes and interactions among themselves and with the environment. This complexity makes unraveling the genetic basis of quantitative traits one of todays eminent challenges. High temperature growth (Htg) in yeast is a quantitative trait and our previous research identified regions in the genome (QTLs) that contain, yet unidentified, Htg genes. In this study, we focused on one QTL with a mild Htg effect, identified the causative genes in it and studied their mode of contribution. By eliminating the effects of other QTL and by using three recombinant strains, we identified seven new Htg genes inside this one QTL. Our experimental design enabled overcoming the challenge of identifying genes with mild to small effect on the trait and this strategy can therefore be useful to address this challenge in plants and animals too. Furthermore, the set of genes and their contribution differed among the three strains we used, unraveling epistatic interactions among the genes inside this QTL and with genes in other QTL. Notably, a significant part of the complex genetic architecture belonged to a specific type of epistatic interaction whereby the effects of some causative genes conceal the effect of other causative genes, making the identification of the latter difficult. Finally, for some Htg genes, the candidate causative polymorphisms were synonymous substitutions suggesting probable contribution of post-transcriptional regulation. Taken together, our study confronted some of the major difficulties in identifying quantitative trait genes and demonstrated ways to overcome these hurdles. We significantly increased the number of Htg genes and our understanding of quantitative traits genetics.

461B. Stability and Patterning in Microbial Communities: Lessons from Engineered Yeast Populations and Mathematical Modeling. ***Wenying Shou***[1], *Babak Momeni*[1], *Kristine Brileya*[2], *Matthew Fields*[2]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Montana State University, Bozeman, MT.
Microbial communities play a critical role in human health and the cycling of elements. Cell-cell and cell-environment interactions within communities govern properties of communities. For example, community stability, the ability of a community to return to its original steady state after perturbations, is an important community property. Patterning, the relative spatial positioning of different species within a community, is also a community property often critical for community activity. However, understanding general principles by which interactions affect stability and patterning has been hampered by the multitude of cell-cell and cell-environment interactions as well as environmental variability in natural communities. To examine how ecological interactions between two populations might impact community stability and patterning, we used mathematical simulations and experiments on engineered yeast communities in controlled environments. Specifically, we engineered S. cerevisiae strains to express different fluorescent proteins and to engage in metabolic competitions and/or exchanges. We found that only interactions that strongly benefited at least one population could potentially achieve stable community composition. Furthermore, a unique pattern was generated by interactions that strongly benefited both partners: partners spatially intermixed by appearing successively on top of each other, insensitive to initial conditions and interaction dynamics. Intermixing was also experimentally observed in a methane-producing community in which two species obligatorily cooperated through redox-coupling. In contrast, intermixing was not

observed in competitive communities or communities where only one population benefited from the interaction. Thus when the fitness effects of interactions are the main determinants of community properties, interactions beneficial to at least one population facilitate stable community composition, and interactions beneficial to both populations lead to partner intermixing.

462C. Regulation of biofilm development through the simultaneous activation and repression of functionally distinct extracellular proteins. ***Zhihao Tan***[1,2], *Michelle Hays*[1], *Cecilia Garmendia-Torres*[3], *Gareth A. Cromie*[2], *Amy Sirr*[2], *Eric W. Jeffery*[2], *Patrick May*[4], *Aimée M. Dudley*[1,2]. 1) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 2) Pacific Northwest Diabetes Research Institute, Seattle; 3) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; 4) Luxembourg Center for Systems Biomedicine, University of Luxembourg, Luxembourg.
Many opportunistic human pathogens form highly structured, multicellular communities that are associated with persistent infection and antimicrobial drug resistance. Thus, factors that allow microorganisms to switch between a planktonic, unicellular lifestyle and a sessile, multicellular lifestyle are an important class of potential antimicrobial drug targets. Building on our previous work demonstrating that increased copies of a single gene, *DIG1*, could reduce the structured colony morphology of one strain of *S. cerevisiae*, we performed a low-copy plasmid overexpression screen to uncover novel regulators of biofilm formation. Our screen, together with subsequent gene deletion analysis, identified five additional genes (*HEK2*, *SAN1*, *SFL1*, *TOS8*, and *YHR177W*) that were able to modulate biofilm formation in a copy number dependent manner. Because several of these genes encode known or predicted transcription factors, we sequenced RNA from colonies in strains in which these genes were either deleted or overexpressed. Our results show common RNA expression patterns for colonies in the biofilm state, with the up-regulation of extracellular genes involved in cellular adhesion and structural constituents of the cell-wall, and down-regulation of genes encoding enzymatic cell wall proteins, such as hydrolases and glucosidases. Our analysis also suggested *PHD1* and *SFG1* as potential regulators of these two classes of genes. The effects of deleting members of these two classes of extracellular genes, as well as *PHD1* and *SFG1*, confirmed their roles in biofilm formation. We propose a model in which the coordinated control of similarly localized but functionally distinct proteins allows *S. cerevisiae* to transition from a unicellular to a multicellular growth state.

463A. Interdisciplinary research on anticancer ruthenium complexes links undergraduate courses and improves student learning and confidence. ***Pamela Hanson***[1], *Laura Stultz*[2]. 1) Biology Department, Birmingham-Southern College, Birmingham, AL; 2) Chemistry Department, Birmingham-Southern College, Birmingham, AL.
Integration of research and teaching is well established as a best practice in undergraduate science education, as it has been shown to increase student knowledge and interest in related subject matter. Explicitly interdisciplinary curricula also positively impact students by improving student articulation of cross-disciplinary concepts and increasing awareness of the connections between scientific disciplines. Here we present assessment findings on a project that links biology and chemistry courses through a shared bona fide research project on the anticancer ruthenium complex indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate (III), also known as KP1019. Through the gradualism model for laboratory education, students in the impacted courses begin by learning fundamentally important techniques then apply these skills to increasingly sophisticated scientific questions, ultimately designing and testing their own novel hypotheses. To promote data-sharing between courses, students upload their findings to a wiki. These posts are then used by subsequent students to shape their experimental design, thereby allowing students to see first-hand the iterative, step-wise nature of basic research. Through this work students have made valuable contributions to our knowledge of the chemical nature of KP1019 as well as its impact on the genetic model organism *Saccharomyces cerevisiae*. On anonymous end-of-semester surveys, students self-reported that the research-based labs significantly increased their degree of experience with integrating disciplines and working on problems with no known outcome. With respect to their ability to interpret results, to analyze data, and to write scientifically, students reported gains comparable to those obtained during intensive summer research programs. These results are consistent with the findings from focus groups conducted by an external evaluator. In addition to validating the pedagogical value of integrating research and teaching, this work suggests that virtual collaboration tools, such as wikis, can be used to successfully illustrate the interdisciplinary nature of modern research.

464B. The 15-week PhD: research methods training for MS Biotechnology students through laboratory investigations of yeast cell physiology. ***Robert M. Seiser***. Biological, Chemical and Physical Sciences, Roosevelt Univ, Schaumburg, IL.
Students in Roosevelt University's non-thesis MS program in Biotechnology and Chemical Sciences are required to complete a minimum of one semester of independent research or enroll in a group-based course, Research Methods. An informal review of bioscience research methods courses at Roosevelt and elsewhere revealed that in some cases, students gained extensive practical training but had no opportunity to design an experimental program, while in others, an emphasis was placed on theory, literature review and/or career exploration over practice. To ensure that MS students could learn to do science in the most direct and relevant ways possible, I revised the curriculum of Research Methods around a multi-week individual project in yeast cell physiology. The layout of the course was intended to emulate a doctoral training program and to include both individual and collaborative activities that would motivate students for further study.

Experimental methods included: 1) design of an original experimental program using a diagrammatic, question-answer approach, 2) identification, acquisition and maintenance of *S. cerevisiae* strains from on-site and off-site collections, 3) laboratory tests and other data collection using microscopy, spectrophotometry, metabolic assay, etc., and 4) revision and refinement of experimental tests over an eight-week period. The experimental program was accompanied by writing and other scientific communication activities as appropriate for each stage of the project: 1) review article and bibliography on the topic of interest, 2) composition and revision of a research proposal in grant format, 3) laboratory notebooks and roundtable-style weekly updates, 4) composition and revision of a research paper in standard journal format, 5) poster presentation, and 6) oral presentation in conference format. Complementary professional development activities included feedback from former students and current PhD candidates, discussions of career options for MS and PhD scientists and an introduction to peer review. Formal assessment and informal feedback from students enrolled in the course indicated satisfaction with the format and with the opportunity to conduct a real research project. The use of *S. cerevisiae* as an experimental system allowed for data generation in a relatively short timeframe and for flexibility in scheduling, as the class met only once per week and many students are employed outside of school. Several students chose projects with broader relevance to biotechnology, indicating alignment with learning goals for the MS program as a whole.

465C. WITHDRAWN