

Poster Sessions



Poster Session 1: DNA replication and cell cycle**PS1-1: Strategies to induce mitotic arrest in fission yeast****Nathalia Chica**, Mari Nyquist-Andersen, Sandra López-Aviles*The Centre of Biotechnology of Oslo (BiO), University of Oslo. 0349 Oslo, Norway*

In the cell, cyclin-dependent kinase activity (Cdk) is opposed by protein phosphatases. Timely regulation of both activities is key to bring about cell cycle events in an ordered fashion. Entry into mitosis is fully dependent on cyclin B-Cdk1 activation to drive phosphorylation of mitotic substrates responsible for the maintenance of the mitotic state until chromosome segregation is carried out. By contrast, a switch of the governance of protein kinases over protein phosphatases is required to promote nuclear division and mitotic progression. Besides, feedback loops between cyclin B-Cdk1 and APC/C complex (anaphase promoting complex) determine the control of mitotic exit, since Cdk1 activates the APC/C and this in turn leads to cyclin B degradation. Although, the core components guiding these events have been described, the understanding of how mitotic Cdk phosphorylations are reverted during exit from mitosis, and how this is temporally regulated still require further investigation. We aim at studying the role of Cdk-opposing phosphatases and their regulation during mitotic progression, using fission yeast as model organism. From budding yeast studies it becomes clear that the use of cultures synchronized in metaphase facilitates the analysis of mitotic progression greatly. To set up an efficient method of synchronization, we have taken advantage of the inactivation of Cdc20, an essential activator of the APC/C. The APC-Cdc20 acts in early mitosis initiating the ubiquitination and proteosomal degradation of Securin and Cyclin B, in order to unblock sister chromatid separation and promote Cdk downregulation, respectively. Therefore, its depletion leads to metaphase arrest with unseparated chromatids and high Cdk activity. Here, we will show different conditional strategies to control Cdc20 activity, in order to generate a sustained mitotic arrest under repressive conditions, while supporting normal growth and cell cycle progression under permissive conditions.

PS1-2: Casein kinase I regulates chitin synthase II endocytosis during mitotic exit in yeast**Cheen Fei Chin**, Kaiquan Tan, Foong May Yeong*Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, MD4, 5 Science Drive 2, Singapore*

Cytokinesis is a key step in mitosis in which a dividing cell undergoes physical separation to form two genetically identical daughter cells. In budding yeast, cytokinesis is accomplished by coordinating the contraction of Actomyosin-ring (AMR) with the synthesis of primary septum by a transmembrane cytokinetic enzyme, chitin synthase II (Chs2p). Previous study from our lab demonstrated that Cdc14p dependent dephosphorylation of Chs2p is required for its release from the rough endoplasmic reticulum to the neck to lay down the primary septum. Upon arrival at the neck, Chs2p is removed from the division site via Sla2p-dependent endocytosis. However, the factors that trigger endocytosis of Chs2p for its removal from the neck after cytokinesis are largely unknown. It has been previously shown that an evolutionary conserved kinase, casein kinase I is required for promoting the endocytosis of transmembrane cargoes. Here, we first showed that Chs2p is a cargo of clathrin-mediated endocytosis (CME) by deleting the key components that are involved in this process. We then established the spatio-temporal localization of Chs2p, relative to key CME components, and yeast casein kinase I, Yck2p, at the end of mitosis using fluorescence timelapsed microscopy. We further investigated the role of casein kinase in regulating Chs2p endocytosis at the end of mitosis using a temperature sensitive allele of yeast casein kinase I (*yck1Δ yck2-ts*). *CHS2* mutagenesis was also performed to identify potential residues that are phosphorylated by the Ycks during end of mitosis. Our data implicate Ycks in the removal of Chs2p at the end of mitosis and reveal a role for Ycks in the control of an enzyme involved in cell division.

PS1-3: Coupling of RNA polymerase III assembly and cell-cycle control in *Saccharomyces cerevisiae*

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Little is known about the RNA polymerase III (Pol III) complex assembly and its transport to the nucleus. We demonstrate that a missense *cold-sensitive* mutation *rpc128-1007* in the sequence encoding the C-terminal part of the second largest Pol III subunit, C128, affects the assembly and stability of the enzyme. Moreover, cells harboring *rpc128-1007* mutation have impaired cell-cycle progression. The mutant cells have arrested division as their cells are unbudded with size larger than normal. The analysis by flow cytometry revealed that most *rpc128-1007* cells have a G1 content of DNA. Additionally, mutant cells showed increased sensitivity to alpha-factor—the mating pheromone arresting the cell cycle. We thus conclude that *rpc128-1007* causes cell-cycle inhibition at G1 phase. We previously identified that defect of Pol III assembly in *rpc128-1007* cells is corrected by Rbs1 protein [1]. We now show that increased *RBS1* expression counteracts the *rpc128-1007*-mediated G1 arrest. Furthermore, overexpression of *RBS1* corrects the morphology differences of mutant cells. Contrary cells lacking Rbs1 show a mild delay in exit from G1 phase. These results indicate that Rbs1 protein is involved in both, Pol III assembly and cell-cycle control. This study is supported by the Foundation for Polish Science (Parent-Bridge Programme/2010-2/2) and the National Science Centre (UMO-2012/04/A/NZ1/00052).

[1] Ciesla M. et al. (2015) *Mol Cell Biol.* 35:1169–81.

PS1-4: Cdc48 facilitates recovery from replication stress by determining the fate of Mrc1

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Cells are constantly subjected to endogenous and exogenous sources of DNA damage and are particularly vulnerable during S phase, in which the damage can result in transmission of mutations and other genomic aberrations to daughter cells. To prevent this, cells employ the intra-S phase checkpoint. Activation and inactivation of this replication stress-induced checkpoint is highly regulated but where much is known about the former, the latter has not yet been as studiously described. One of several parallel mechanisms for inactivating the replication checkpoint and resume DNA replication is SCF-Dia2 mediated ubiquitination and subsequent degradation of the checkpoint mediator and fork protection complex component Mrc1. We have previously shown that upon replication stress, ubiquitinated Mrc1 is relocalized to the intranuclear quality control compartment (INQ) where it is subsequently degraded. Here we show that the regulation of Mrc1 during and in recovery from replication stress depends on the proteasome-associated chaperone Cdc48. Lack of Cdc48 during and in recovery from replication stress causes impaired degradation of Mrc1 and persistent localization to INQ resulting in delayed replication completion. Our data suggest a model in which Cdc48 determines the fate of INQ-localized Mrc1 after replication stress, committing the ubiquitinated protein to proteasomal degradation while also possibly promoting the recycling of a de-ubiquitinated pool to the replication fork where it is required for efficient restart and completion of replication upon checkpoint recovery.

PS1-5: Structure/function analysis of the Hif1 histone chaperone in *Saccharomyces cerevisiae*

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Understanding how eukaryotic cells assemble their chromatin is a significant research subject in part because several human pathologies including cancer are associated with defects in chromatin assembly. Transporting of newly synthesized histones H3/H4 occurs in a stepwise fashion and is regulated by a variety of protein factors including histone chaperons. In the budding yeast *Saccharomyces cerevisiae*, the Hif1 protein is an evolutionarily conserved H3/H4-specific histone chaperone and a member of the nuclear Hat1 complex that catalyzes the deposition-related acetylation of newly synthesized histones H4. Hif1 as well as its human homolog NASP have been implicated in an array of chromatin-related processes including histone H3/H4

transport, chromatin assembly, DNA repair and telomeric silencing. In this study, we elucidate structural and functional aspects of Hif1. Through targeted mutational analysis, we demonstrate that the acidic region of yeast Hif1 which interrupts the TPR2 is essential for physical interaction with the Hat1-complex. We also demonstrate that Hif1 requires its C-terminal basic patch for nuclear localization. Furthermore, we provide evidence for the involvement of Hif1 in regulation of histone metabolism by showing that cells lacking *HIF1* are both hypersensitive to histone H3 overexpression. Finally, we describe a functional link with a transcriptional regulatory protein Spt2 possibly linking Hif1 to transcription-associated chromatin reassembly. Taken together, our results provide novel mechanistic insights into Hif1 functions and establish it as a key player in various chromatin-associated processes.

PS1-6: Mechanism of DNA synthesis during BIR

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DSBs that present only one end for repair (for example replication fork collapse, telomere erosion or segregation of truncated chromosomes) are repaired by strand invasion into a homologous duplex DNA followed by replication of several kb to the chromosome end through a mechanism named break-induced replication (BIR). BIR can be detrimental if it occurs between genomic dispersed repeats because it can generate gross chromosome rearrangements, such as non-reciprocal translocations and copy number variation, a distinguishing feature found in several types of cancer. BIR shares in common with gene conversion the first steps, including DSB resection and Rad51-dependent invasion of homologous sequence, the uniqueness is that during BIR, a reparative DNA synthesis of both leading and lagging strands proceeds to the end of the donor chromosome resulting in extensive loss of heterozygosity (LOH). Here we describe an *in vivo* chromosomal system that permits the induction of a single one-ended DSB, allowing the study of BIR by genetic and physical approaches. Using this system, we recently showed that DNA synthesis during BIR is conservative, in contrast to replication observed in S-phase. Other groups have shown that leading strand synthesis during BIR involves a “migrating bubble” resulting in the accumulation of extensive ssDNA protruded from the D-loop. This observation raises the intriguing possibility that lagging strand synthesis is asynchronous and not governed by the same principles and factors involved during a physiologic DNA replication or two-ended DSB repair. We assume second strand synthesis requires DNA Pol α -primase as this is the only polymerase known to initiate DNA synthesis de novo. Using a temperature-sensitive allele of DNA-primase (*pri2-1*) we detected BIR initiation by primer extension PCR, but no BIR products by Southern blot of digested genomic DNA, which requires double-stranded DNA to be cut by restriction endonucleases. Thus, Pol α complex is required for BIR completion while it is dispensable for BIR initiation. We are currently investigating how Pol α complex is recruited on the nascent ssDNA and the nature of second strand synthesis, which can occur in one or multiple reactions.

PS1-7: The spindle checkpoint protein Mad2p is a new factor implicated in origin firing in case of replication stress in *S. cerevisiae*

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During mitosis, the protein Mad2 is a key component of the spindle checkpoint which allows the proper segregation of chromosomes into the two daughter cells. However, the function of this protein outside of the mitotic checkpoint is quite elusive. Here we show that Mad2p is also important to face the slowing down of the replication fork in S-phase in case of replication stress. Using 2D gel analysis and BrdU immunoprecipitation, we have shown that MAD2 deletion affects centromere replication and early and late origin firing. Mad2p acts on replication synergistically with the intra-S checkpoint effector rad53, but independently of the DNA damage checkpoint. The contribution of Mad2p during replication is independent of the other members of the spindle checkpoint (Mad1p, Mad3p, Bub3p) arguing that the function of Mad2p is independent of its role in the spindle checkpoint. However, restricted expression of Mad2p during mitosis can rescue all the replication defects observed in the absence of Mad2p. All together, these observations suggest that Mad2p favor the recruitment of replication factors in M phase in addition of its fundamental role in the spindle checkpoint.

PS1-8: Global Analysis of molecular fluctuations associated with cell cycle progression in *Saccharomyces cerevisiae*

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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. By combining Synthetic Genetic Array (SGA) technology with high-throughput fluorescence microscopy of the ORF-GFP fusion collection, we have generated image-based data for ~75% of the yeast proteome. Our strategy involves scoring diagnostic fluorescent markers that indicate cell cycle position, which permits the computational classification of yeast cells into one of six predetermined cell cycle stages, and subsequently quantifying protein abundance and localization for each member of the GFP collection. We automated cell cycle classification using a supervised neural network-based approach that functions with ~97% accuracy. With mean GFP-pixel intensity as a metric for protein abundance, we determined how the entire visible budding yeast proteome fluctuates over the course of the cell cycle. We are also adapting our neural network classification method for the automated assignment of GFP-fusion proteins to 21 different subcellular compartments. When combined with cell cycle transcriptional information, this unique platform will provide a resource 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. On a broader scale, our dataset will allow us to study pre- and post-translational gene regulation in an ordered and highly conserved biological process, providing a unique opportunity that is not possible with existing eukaryotic data.

PS1-9: Biochemical and structural characterization of the microtubule- associated protein Irc15

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The yeast genome contains 68 genes (1,1% of all yeast proteins) which encode for flavin-dependent proteins. Thirty-five flavoproteins require FAD (74%) and fifteen require FMN (26%). This utilization of FMN and FAD is similar to the distribution across all kingdoms of life. Several yeast flavoproteins could serve as convenient model systems, nevertheless many yeast flavoenzymes are poorly characterized. Biochemical properties such as substrate specificity, kinetic parameters and reaction partners need to be determined to improve our understanding of human orthologs [1]. One flavoenzyme, which raised our interest is Irc15p (increased recombination centers 15). This protein is similar to FAD-containing lipoamide dehydrogenases, however, it lacks the internal dithiol-disulfide motif that is involved in the oxidation of lipoamide in the pyruvate dehydrogenase complex. Interestingly, Irc15p was found to be associated with microtubules and displayed an influence on the dynamics of microtubules. Loss of Irc15p function resulted in delayed mitotic progression due to the failure to establish tension between sister kinetochores [2]. We are currently investigating the biochemical properties of recombinant Irc15p to better understand its impact on microtubules and its role in mitosis.

[1] Gudipati V, Koch K et al. (2014) *Biophys. Acta* 1844, 535-544; [2] Keyes BE and Burke DJ (2009) *Current Biology* 19, 472-478.

PS1-10: The roles of PCNA postranscriptional modifications in modulating of intra-chromosomal homologous recombination pathways

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Mms2 and Rad5 are responsible for polyubiquitination of PCNA. This modification initiates the error-free DNA damage tolerance mechanism based on transient template switch when replication complex encounters DNA

lesion. The mechanistic role of PCNA polyubiquitination in this process is far from being understood. To better recognize the roles played by PCNA polyubiquitination in replication fork we analyzed how the Mms2 and Rad5 influence intra-chromosomal recombination between direct repeats separated by over 5 kb. Our results show that deletions of *MMS2* and *RAD5* limit frequency of gene conversion and that these genes function epistatically. This demonstrates that polyubiquitination of PCNA stimulates spontaneous gene conversion between direct repeats. In similarity to situation previously described for inverted repeats recombination, we also noticed a weak stimulatory effect of PCNA polyubiquitination on Rad51/Rad59-independent, replication dependent, single strand annealing (SSA). In general the results point to prorecombinogenic activity of PCNA polyubiquitination. Additionally, Rad52/ Rad59-dependent and Rad51-independent recombination between the direct repeats, via SSA, is not affected by Mms2 defect, but it is moderately enhanced by deletion of the *RAD5* gene, suggesting the role of polyubiquitination independent activity of Rad5 in limiting of spontaneous SSA between direct repeats. The role of PCNA SUMOylation in modulating the frequency of different recombination pathways will be discussed.

PS1-11: P bodies regulate transcriptional rewiring during replication stress

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P bodies are RNA-protein granules that form in the cytoplasm of eukaryotic cells in response to various stresses and are thought to serve as sites of storage of mRNAs before degradation or translation. We recently discovered that P bodies form, in yeast, in response to replication stress induced by HU (hydroxyurea), an anti-cancer drug that inhibits dNTP synthesis and slows down replication. We also showed that P body components are required for cell survival of replication stress as mutants lacking key P body components *Lsm1*, *Pat1* and *Dhh1* are strongly sensitive to HU. Together, these data suggest that P bodies are part of the post-transcriptional regulation network during replication stress. Here, we aimed to identify mRNAs that are processed by P bodies during replication stress. First, using an SGA-based suppressor screen we identified a core set of 53 genes which, when inactivated, suppress *lsm1Δ* and *pat1Δ* HU sensitivity, suggesting that their expression during replication stress is toxic and might be regulated by P bodies. Interestingly, this set of suppressors is enriched for genes implicated in RNA metabolism and transcription, highlighting the need for tight regulation of these processes during replication stress. Second, we performed a transcriptome study on *lsm1Δ* upon acute HU exposure in order to identify mRNAs that are stabilized in the absence of P bodies and consequently might be potential targets for P body-dependent degradation. We found that the transcriptome in *lsm1Δ* is altered both during normal growth and during replication stress, with more than 600 genes being differentially expressed in *lsm1Δ* compared to wild-type. Among the differentially expressed genes, we identified the transcription repressor Yox1 as a potential interesting target, as *YOX1* mRNA is up-regulated in *lsm1Δ* and deletion of *YOX1* suppress *lsm1Δ* and *pat1Δ* HU sensitivity. Furthermore, we found that Yox1 targets for transcriptional repression are enriched in the genes that are down-regulated in *lsm1Δ* and that *YOX1* overexpression toxicity is HU- as well as *LSM1*-/*PAT1*-dependent. Finally we have preliminary evidence showing that *YOX1* mRNA localizes to P bodies. Taken together, these data suggest that *YOX1* mRNA is degraded in a manner dependent on P bodies during replication stress and that this degradation might be required to prevent repression of Yox1 targets expression. Our current efforts aim at identifying Yox1 targets during replication stress.

PS1-12: Programmed cell death is the fate for the majority of the progeny after a yeast mitotic catastrophe provoked by Topoisomerase II deficiency

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The main function of topoisomerase proteins is to disentangle DNA in all its biological processes. Topoisomerase II (Top2) is an essential protein because of its unique ability to cut double stranded DNA and resolve catenations. When the Top2 gene is deactivated the cell division suffers from a massive entanglement of the chromosomes which results in Anaphase Bridges (ABs) that cannot be resolved and that leads, in most cases, to the death of both daughter cells [1]. We have studied through fluorescence videomicroscopy the evolution of the first cell cycle in strains that carry several thermosensitive *top2* alleles. We have found that some cells

struggled to resolve ABs during the cell division for several hours whereas others quickly severed the AB. We will show that these phenotypes correlate to: i) the top2-ts mutant used, ii) the cell cycle stage the cell is at the time of top2-ts inactivation, and iii) the presence of other secondary mutations for checkpoint and repair systems. In addition to filming the first cell cycle, we have monitored any other cell cycle beyond and found that *top2-ts* mutants seldom enter a second cell cycle. However, this situation can be surpassed when the Rad9-mediated DNA damage checkpoint is abolished, indicating that daughter cells coming from a mitotic catastrophe get blocked in G1 due to massive DNA damage. Interestingly, another factor that restrained the entry into a new S-phase was the metacaspase Yca1/Mca1. Supporting a role for programmed cell death as an impediment for cell cycle progression after mitotic catastrophe, daughter cells eventually lost mitochondrial membrane potential, suffered from reactive oxygen species (ROS) accumulation, lessened their metabolism, and ended up dying with morphological hallmarks of apoptosis/necrosis. We propose that yeast can sense mitotic catastrophe and trigger, in many instances, programmed cell death to quickly get rid of unviable progeny. This work has been supported by Instituto de Salud Carlos III (PI12/00280 to F.M.) and La Laguna University through the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (predoctoral fellowships TESIS20120109 to C.R.) All these programs were co-financed with the European Commission's ERDF structural funds. [1] Holm C., Goto T. et al. (1985) *Cell*, 41,553–63.

PS1-13: Towards a platform for global mapping of binary protein interactions under diverse conditions

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Protein-protein interaction network maps have been vital to our understanding of cellular systems. However, networks mapped via these approaches are inherently static. While network dynamics owing to transcript-level changes can be inferred, no existing global binary interaction method fully captures comprehensive network dynamics, e.g., post-translational effects of regulation and environmental change. Here we combine a conventional Yeast two-hybrid (Y2H) assay with flow cytometry and Barcode Fusion Genetics (BFG-Y2H) technology. Preliminary fluorescence reporter results demonstrate high assay sensitivity and a quantitative output. Unlike reporters in current global methods, it can be applied to non-dividing, e.g., cell-cycle arrested, cells. The described “BFG-GFP-Y2H” approach is compatible with genome-scale interaction mapping across multiple conditions within a single experiment.

PS1-14: The transcriptional activity of Sfp1 regulates cell growth and division

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Proper regulation of cell growth is a primarily concern of all living organisms. A fundamental engine of cell growth is ribosome biogenesis, a remarkably energy-intensive process tightly regulated at the transcriptional level in the budding yeast *S. cerevisiae*. Sfp1 (Split Finger Protein 1) is a stress- and nutrient-sensitive transcription factor involved in this control. Early DNA microarray experiments performed in cells where SFP1 is under control of the galactose-inducible GAL1 promoter suggested that Sfp1 is a positive regulator of a broad set of genes involved in cell growth, including ribosomal protein genes (RPGs) and ribosome biogenesis (Ribi) factors, but also tRNA synthetases, translational initiation and elongation factors, and nucleotide biosynthesis genes. Moreover SFP1 is a potent regulator of cell size: *sfp1Δ* cells have extremely reduced cell size compared

to WT, whereas overexpression of SFP1 increases cell size. Interestingly *sfp1Δ* cells are impervious to carbon source mediated size adaptation, and when cells are grown in poor carbon sources *Sfp1* nuclear concentration is reduced. This suggests that *Sfp1* could be important to reach the maximum growth potential by sustaining transcription of growth-related genes. Here we show results of ChIP-seq experiments under conditions of normal and elevated SFP1 expression and after conditional nuclear depletion of *Sfp1* by the Anchor-away technique. These experiments reveal that *Sfp1* is an unusual type of TF whose promoter binding is not readily detected at all of its target genes. Examples of its transcriptional activity are given for 2 specific gene classes: RP and Ribi genes. Moreover initial evidences for a possible transcriptional role of *Sfp1* on G1 cyclins transcription are described, suggesting that this protein could be important for the “feedback-first” regulation that precedes DNA replication.

PS1-15: Role the signaling pathway Greatwall kinase during cell cycle progression in budding yeast

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Cell cycle progression depends on the phosphorylation of target proteins by cyclin-dependent kinases (Cdk) followed by their dephosphorylation by phosphatases (e.g. PP2A). The regulation of protein kinases during cell cycle has been largely reported, whereas the control of phosphatases has lagged far behind. Several recent studies identified a new signaling pathway named Greatwall kinase (Gwl), conserved from yeast to man, which controls the activity of the phosphatase PP2A-B55. It was shown that this pathway controls mitotic entry and progression in metazoans, whereas it promotes entry into quiescence upon TORC1 inactivation in yeast. Surprisingly despite its strong evolutionary conservation, loss of Rim15 (Gwl) or its substrates (Igo1/2) seemed to have no effect on cell cycle progression in *S. cerevisiae*, at least when cells are grown in rich medium. We reasoned that this maybe due to culture conditions that favor kinase over phosphatase activities. To uncover a potential role of the Rim15/Gwl pathway on cell cycle progression, we used two conditions: i) artificially lowering Cdc28 kinase activity and ii) using growth-limiting media containing sub-optimal amounts of leucine - a positive regulator of TORC1 - in which Igo1 is highly phosphorylated (i.e. the pathway is activated). We also developed a novel assay, based on fast EdU (5'-ethynyl-2'-deoxyuridine) incorporation and bivariate FACS analysis, to identify more precisely cells in S phase. In these growth-limiting conditions, we found that the Rim15 pathway is required for a timely entry into the cell cycle (Start) as well as for S-phase entry. We then identified Cln2, one of the three G1 yeast cyclins, as a target of the Rim15 pathway. Dephosphorylation of Cln2 leads to its stabilization and accumulation in small G1 cells, causing precocious Start and S-phase entry. As similar defects promote tumorigenesis in mouse and humans, targeting the Gwl pathway, instead or in addition of Cdks, may prove useful to treat cancer.

PS1-16: The participation of Dpb2, a noncatalytic subunit of DNA polymerase epsilon, in maintaining microsatellite stability

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The multi-subunit DNA polymerase epsilon holoenzyme (Pol ε HE) of *Saccharomyces cerevisiae* consists of four subunits: Pol2p, Dpb2p, Dpb3p and Dpb4p, of which Pol2p and Dpb2p are essential for cell viability. Except for the catalytic Pol2p subunit, the role of the other three auxiliary subunits is not well defined. Several mutants in *dpb2* allele previously isolated in our laboratory are mutators and show temperature-sensitive phenotype as well as altered protein-protein interactions between Pol2p and mutated forms of Dpb2p. Pol ε interacts by Dpb2p with the Cdc45p-MCM-GINS complex (CMG). CMG, as a replicative helicase, unwinds the DNA duplex allowing the movement of the replication fork. It may be responsible for targeting of Pol ε to the leading strand. Also, the interaction of GINS subunits, Psf1 and Psf3, with Dpb2p, required for the assembly of CMG during the initiation of DNA replication, helps to integrate Pol ε into the replisome. Using yeast two hybrid system we have observed a significant reduction in the protein-protein interaction between Psf1p/Psf3p subunits of the GINS complex and mutated Dpb2p subunits. Our work is related to the mechanisms and sources of microsatellite instability. Although the explanation of the phenomenon of microsatellite DNA sequences

instability is now the subject of intensive research, so far any reports has been shown on the participation of non-catalytic subunits in maintaining the stability of tandem repeats of DNA. We try to answer the question whether the decreased stability of Pol ε HE in *dpb2* mutant strains and/or impaired interactions with other replisomal components affect the stability at short tandem repeats. We assume that Dpb2p, by stabilizing the holoenzyme and interactions with the CMG complex, could indirectly prevent mutations during DNA replication. Increased dissociation of a less stable Pol ε may significantly increase polymerase slippage on DNA. In our work we have tested this hypothesis using the *dpb2* strain from our laboratory and plasmids specially designed and constructed at Thomas Petes's laboratory. For all microsatellite DNA sequences tested, we have observed an increase in spontaneous mutagenesis.

Poster Session 2: Gene expression: from epigenetic regulation to mRNA stability

PS2-1: Insights into the tRNA-derived small RNAs biogenesis in *Saccharomyces cerevisiae*

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In the past years, it became evident that the correlation between mRNA and protein abundances in cells is surprisingly poor. Studies measuring the transcriptomes and proteomes of cells demonstrated that for the vast majority of protein coding genes, the transcript levels do not reflect the actual protein levels. Most of the differences could be explained by translation regulation control mechanisms. We have recently described a new possibility of protein biosynthesis regulation by the direct interaction of small noncoding RNAs with *Saccharomyces cerevisiae* ribosomes [1]. These RNAs are called rancRNAs (ribosome-associated noncoding RNAs). We were able to detect and confirm by independent experimental methods multiple novel stable RNA molecules differentially processed from well-known ncRNAs, like rRNAs, mRNAs, tRNAs or snoRNAs, in a stress-dependent manner. We further demonstrated that the mRNA-derived rancRNA is needed for rapid shutdown of global translation and efficient growth resumption under hyperosmotic conditions [2]. Currently our interest is focused on revealing the potential of tRNA-derived small RNAs as specific regulators of protein biosynthesis. Here, we present the comprehensive analysis of tRNA processing in *S. cerevisiae* to short stable RNAs called tRFs (tRNA-derived fragments). In our study we have used the northern blot method, which provides the robust estimation of the tRFs size, independent from numerous tRNA modifications which could interfere with reverse transcription. Moreover we show that different RNA isolation methods significantly vary in tRFs recovery. With the employment of the optimized protocol, we were able to identify tRFs derived from all yeast tRNA isoforms. However, in contrary to previous data, we did not observe significant stress-dependent changes in their accumulation. Interestingly, most of the observed tRFs were shorter than halves, suggesting the presence of yet unknown biogenesis pathway. We also provide the first evidence that 3'-tRFs are as abundant as the 5'-one. The resulting set of *S. cerevisiae* tRFs provides a robust basis for further experimental studies on their biological functions.

[1] Zywicki M, Bakowska-Zywicka K, Polacek (2012) *Nucleic Acids Res.* 40, 4013-24; [2] Pircher A, Bakowska-Zywicka K et al. (2014) *Mol Cell.* 54, 147-55.

PS2-2: Functional analysis of EAF protein in *Schizosaccharomyces pombe*

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Eukaryotic gene expression is regulated by RNA polymerase II, in association with an array of proteins. For a long time, research was focused on initiation of transcription as the major regulatory step during gene expression. However, transcription elongation has recently been recognized as another important step controlling expression of genes. ELL (Eleven Nineteen Lysine Rich Leukemia) and EAF (ELL associated factor) family of proteins have been shown to be components of different elongation complexes governing the activity of RNA polymerase II. Most of the studies on these proteins have focussed on understanding the functions of the ELL protein, whereas the functions of the EAF protein still remain to be elucidated in detail. In this study, we have initiated experiments to characterize the EAF protein in *Schizosaccharomyces pombe*. Our data shows that deletion of EAF decreases the growth of yeast cells under optimum growth conditions. Furthermore, EAF null mutant exhibits reduced viability upon exposure to DNA damaging agents. We next aligned the EAF sequences from various organisms, and delineated two sequences that showed a high degree of conservation. Subsequently, truncation mutants were generated and their ability to rescue different phenotypes associated with deletion of EAF was tested. It was observed that the carboxyl terminal region of the EAF protein was indispensable for the growth of cells under optimum growth conditions as well as under DNA damaging conditions. We further determined if the human EAF protein could complement the absence of EAF in *S. pombe* and if it could interact with *S. pombe* ELL. Our results show that human EAF can not compensate for the lack of EAF in *S. pombe* and also, unable to interact with the *S. pombe* ELL. Taken together, our results provide new functional insights into the *S. pombe* EAF protein.

PS2-3: Methanol-induced genes in methylotrophic yeast *Hansenula polymorpha* DL1: Features of genome organization and expression in a popular yeast cell factory

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H. polymorpha is methylotrophic yeast widely used for studies of methanol metabolism, peroxisome biogenesis and function, as a cell factory for production of recombinant proteins and metabolic engineering. We have reported previously complete sequences of *H. polymorpha* DL1 nuclear and mitochondrial genomes, phylogenetic gene content and gene order analysis, identification of subtelomERICally biased protein families in *H. polymorpha* and potential centromeres marked by clusters of LTR elements at G + C- poor chromosomal loci, data on evolution of MUT pathway genes in yeast and fungi (Ravin et al., BMC genomics, 2013,14: 837). The performed genome-wide RNA-seq analysis of *H. polymorpha* transcriptome obtained from methanol and glucose grown cells is now supplemented by the analysis of patterns of chromosomal distribution and of methanol-induced genes (MIGs), examination of their promoter sequences, classification of MIGs to different functional categories. We have found, that in general MIGs are evenly scattered throughout the genome and rarely forms clusters. Short MIG clusters (up to 10 genes) do exist and within these clusters MIGs tend to organize in operon-like structures with very short intergenic regions. MIGs are absent from regions proximal to potential centromeres, thus avoiding potential silencing by centromeric heterochromatin. Bioinformatic analysis of promoter regions of highly expressed MIGs have revealed enrichment for binding sites of known transcriptional regulators, as well as presence of several new motifs, potentially important for MIG regulation. GO analysis have shown, that besides known categories of genes involved in methanol metabolism, peroxisome biogenesis and function etc., genes involved in DNA repair are also moderately up-regulated in methanol-grown cells. This observation allows to hypothesize that growth on methanol may be accompanied by increase in mutation rate indicative of elevated degree of oxidative DNA damage in methanol-metabolizing cells.

PS2-4: Phenotypic heterogeneity guides adaptive evolution

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Genetically identical cells frequently display substantial heterogeneity in gene expression, cellular morphology and physiology and such phenotypic variation is occasionally transmitted across cell generations. It has been suggested that by rapidly generating a sub-population with novel phenotypic traits, phenotypic heterogeneity accelerates the rate of adaptive evolution in populations facing extreme environmental challenges. This issue is important as cell-to-cell phenotypic heterogeneity may initiate key steps in microbial evolution of drug resistance and cancer progression. Here, we study how stochastic transitions between cellular states influence evolutionary adaptation to a stressful environment in yeast *Saccharomyces cerevisiae*. We developed inducible synthetic gene circuits that generate varying degrees of expression stochasticity of an antifungal resistance gene. We initiated laboratory evolution experiments with genotypes carrying different versions of the genetic circuit by exposing the corresponding populations to gradually increasing antifungal stress. Phenotypic heterogeneity enhanced the adaptive value of beneficial mutations through synergism between cell-to-cell variability and genetic variation. Remarkably, phenotypic heterogeneity evolved rapidly under directional selection pressure. We conclude that phenotypic heterogeneity shapes evolutionary trajectories and facilitates evolutionary rescue from a deteriorating environmental stress.

PS2-5: Promoter architecture and transcriptional activation of Abf1-dependent ribosomal protein genes in *Saccharomyces cerevisiae*

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Ribosome biogenesis, the most energy-consuming process in the cell, is tightly regulated in function of metabolic needs and growth rate. In *Saccharomyces cerevisiae*, ribosome biogenesis is mainly regulated at the transcriptional level and it requires the coordinated expression of more than 750 genes coding for both proteins and RNA molecules [1]. Among them, 79 ribosomal proteins (RP), together with 4 rRNAs, represent the essential structural components of mature yeast ribosomes. Due to genomic duplication, budding yeast RPs are encoded by 138 genes, most of which require the general regulatory factor Rap1 for transcription. A minority of RP genes, however, appear to be Rap1-independent and to require instead Abf1. While the promoter architecture and transcriptional regulation of Rap1-dependent RP genes has been extensively studied [2], much less is known about the small subset of RP genes whose promoters are demarcated by an Abf1 binding site. By *in silico* analysis, we found that Abf1-containing *RPL3*, *RPL4B*, *RPP1A*, *RPS22B*, *RPS28A* and *RPS28B* promoters share a common architecture, in which a single Abf1 binding site is followed, at a very short distance, by a strongly conserved sequence motif matching the predicted binding site for Fhl1, an essential and specialized regulator of RP gene transcription. Subsequent *in vivo* (ChIP) and *in vitro* (EMSA) analyses confirmed the association of Abf1 and Fhl1 proteins with their corresponding binding sites at some of these promoters. Promoter mutational analysis showed that Abf1 binding is generally required for full RP gene transcription under optimal growth conditions, suggesting a possible involvement of Abf1 in Fhl1 recruitment to these promoters. Remarkably, however, these genes show different responses to the same Abf1/Fhl1 site mutations within their promoter, probably as a consequence of subtle effects of transcription factor binding site distance and orientation. In the particular case of *RPS22B*, whose second intron hosts the snoRNA gene *SNR44*, an additional Tbf1 binding site appeared to play a key role in promoter function, influencing *RPS22B* and *SNR44* expression to different extents. [1] Bosio MC, Negri R, Dieci G (2011) *Transcription* 2, 71-77; [2] Knight B, Kubik S, Ghosh B et al (2014) *Genes Dev* 28, 1695-1709.

PS2-6: An autoregulatory positive transcriptional feedback mediated by Rlm1 is essential for development of the Slt2 MAPK dependent gene expression program

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Signal transduction MAPK pathways must be precisely regulated in order to modulate specific adaptive responses. In consequence, there are many regulatory mechanisms to modulate signaling through these pathways. Negative feedbacks contribute to attenuate responses whereas positive feedbacks should amplify signaling. For the CWI pathway, not only the MAPK of the pathway, Slt2, but also the related pseudokinase Mlp1 and the transcription factor Rlm1 are transcriptionally induced under cell wall stress conditions in an Rlm1 dependent manner, pointing to the existence of feedback regulatory circuits. In this work, we have demonstrated that the transcription factor Rlm1 exerts, under cell wall stress conditions, a transcriptional positive feedback mechanism on the expression of both *SLT2* and *RLM1* itself, through interactions with the Rlm1 binding sites present at the promoter regions of both genes. By site-directed mutagenesis of Rlm1 binding domains at *SLT2* and *RLM1* promoters, we created yeast strains with individual specific blockades of both positive feedbacks. CWI signaling, measured by quantification of the MAPK Slt2 phosphorylation, was not impaired in any of the two strains respect to the wild-type strain. However, abrogation of the feedback of Rlm1 on itself results in a severe impairment in the transcriptional activation of genes regulated through the CWI pathway whereas the increase in the amount of Slt2 mediated by the activity of Rlm1 in the other positive transcriptional feedback exerted by Rlm1 on *SLT2*, although partially contributing to the CWI gene expression levels, has less impact on the CWI output response. Therefore, phosphorylation of Rlm1 by the MAPK Slt2 is necessary but not sufficient for a functional CWI transcriptional response, being the positive feedback of Rlm1 on itself, activated by the

stress, essential for a proper transcriptional adaptive response.

PS2-7: The role of the Mlp1 in genomic stability

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During transcription, negative supercoils accumulate behind the advancing RNA polymerase and it facilitates the unwinding of the DNA helix. This transient DNA opening favours the annealing of the nascent mRNA to the transcribed strand (TS) to form R-loop. Within R-loops, the displaced non-transcribed strand (NTS) remains single-stranded and it is more susceptible to be damaged. R-loop formation occurs naturally as an intermediate in specific process and account, at least partly, for the increased DNA damage on the NTS of transcribed genes. This has been observed in *E.coli*, yeast and human cells and contributes to transcription-associated recombination. We are interested in understanding the mechanisms associated with mRNA particle biogenesis and the control of R-loops formation. Using *Saccharomyces cerevisiae* as a model organism, we have found MLP1 in a screening for deletions of non-essential nuclear genes that show R-loop dependent hyper-recombination phenotype. *MLP1* encodes a nuclear pore basket protein that plays an important role in unspliced mRNA retention, SUMO regulation and telomere organization. In addition, gene gating defects has been proposed in *mlp1*^Δ. We currently investigate the role of *Mlp1*, and the nuclear localization of transcribed genes in preventing genomic instability. Our ongoing results will be presented and discussed.

PS2-8: R-loop-mediated genome instability in histone mutants

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R-loops, nucleic acid structures consisting of an RNA-DNA hybrid and a displaced single-stranded (ss) DNA, are a potential source of genomic instability, but they also play an important role in bacterial replication and immunoglobulin class switching. Among the different mechanisms proposed to explain R-loops mediated genome instability, an important one is its potential to block replication forks. We have shown that R-loops are linked to chromatin condensation and that the chromatin reorganizing complex FACT is required for replication fork progression through transcribed DNA. In order to explore the possible role of specific histone residues in R-loop stabilization, we undertook the characterization of specific histone mutants with an R-loop-dependent hyper-recombination phenotype. We are currently investigating the mechanism promoting R-loop accumulation in these mutants and their consequences in genome instability.

PS2-9: RAS/PKA signaling pathway is involved in regulation of RNA polymerase III transcription mediated by TFIIIC factor

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Regulation of RNA polymerase III (Pol III) in yeast *Saccharomyces cerevisiae* in response to carbon source is mediated by general Pol III - repressor, Maf1. Upon a shift of yeast cells from glucose rich media to a medium with a non-fermentable carbon source the Pol III occupancy on tRNA genes was markedly decreased. Surprisingly, that shift also decreased the Pol III occupancy on tRNA genes in a strain lacking Maf1 protein. This result indicates Maf1-independent regulation of Pol III association with tDNA in response to glucose availability. Assuming that RAS signaling pathway is likely to be involved in this process we examined Pol III occupancy and tRNA transcription in *cdc25* mutants. Cdc25 is activator of Ras1/2 GTPases which functions as GTP/GDP exchange factor (GEF) and in consequence activates protein kinase A (PKA), which is a main positive growth regulator in yeast. Decrease of Pol III occupancy and repression of tRNA transcription by a non-fermentable carbon source were reproducibly attenuated in both, *maf1*^Δ and *cdc25-1* single mutants and this effect was surprisingly further enhanced in the double *cdc25-1maf1*^Δ mutant. These results demonstrate that Pol III is controlled by Cdc25 and/or PKA in Maf1-independent manner. According to our and published data, Cdc25 physically interacts with Mds3 (*mck1*^Δ dosage suppressor), a negative regulator affecting sporulation, but not with Pmd1 (paralog of Mds3). Moreover, Tfc3 (part of the TauB subunit of TFIIIC), is predicted to be

phosphorylated by PKA at three serine residues. We showed, that the level of primary tRNA transcript is decreased when cells lack both, Mds3 and Pmd1. Additionally, tRNA transcription is still attenuated when Mafl is deleted in *Δmds3Δpmd1* strain. In contrast, expression of Tfc3 with inactivated PKA - phosphorylation sites unexpectedly results in stimulation of tRNA synthesis, suggesting that PKA-dependent Tfc3 phosphorylation negatively influences Pol III transcription. These results support the hypothesis, that Mafl and Pol III are controlled by separate branches of the RAS signaling pathway and its modulators. This study is supported by the National Science Centre (UMO-2012/04/A/NZ1/00052).

PS2-10: The role of histone acetylation in modulating G1-S cell cycle transcription

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Transcription at the G1/S transition of the cell cycle drives the commitment to cell division and is required for genome integrity maintenance. Deregulation of G1/S transcription is observed in all types of cancer. The mechanism of G1/S transcriptional regulation is evolutionary conserved from yeast to human. In *Saccharomyces cerevisiae*, G1/S transcription is regulated by two distinct transcription factor complexes: SBF and MBF. Whilst SBF and MBF targets have similar temporal patterns of expression the mechanisms of regulation are distinct. SBF is a transcriptional activator whose activity is inhibited by Whi5 in early G1 phase. MBF is a transcriptional repressor and acts together with co-repressor Nrm1 outside of G1 phase. The role of these transcriptional regulators is well established, but how G1/S transcription is regulated at the chromatin level remains largely unknown. Histone modifications can change chromatin state affecting transcription. Here we investigate the role of histone acetylation in G1/S transcriptional regulation. Acetylation of histones results in chromatin relaxation, which is associated with activation of transcription. Our preliminary data show that histone acetylation at SBF and MBF target promoters reaches its maximum upon G1/S transition, which coincides with transcriptional activation. Previous work has established that both histone deacetylase Rpd3 and histone acetyltransferase Gcn5 are recruited to G1/S promoters. However, our results show no significant difference in levels of G1/S transcription in *rpd3Δ* and *gcn5Δ* in asynchronous cultures and G1/S target genes are still cell cycle regulated that in cell cycle synchronized cultures. However, in *gcn5Δ* cells transcription does not reach the maximum peak levels indicating that Gcn5 is necessary for full induction of SBF and MBF target genes. In line with these results histone acetylation at G1/S target promoters depends on Gcn5. In addition deletion of Rpd3 leads to faster cell cycle progression suggesting a biologically significant increase in G1/S transcription. In conclusion, whilst histone acetylation/deacetylation is not required for G1/S cell cycle transcriptional regulation it has a likely role in modulating absolute transcript levels, which is important for cell cycle dependent processes.

PS2-11: Rpb5 mediates RNA polymerase II transcription elongation by modulating its processivity and its interaction with Spt5

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The specific role of Rpb5 in transcription, a common subunit the three eukaryotic RNA polymerases, is not still well established, although we reported that mutations in *RPB5* affect all three RNA polymerases. In addition, our data and those of others suggest the participation of Rpb5 in concerted association with Rpb1 and Rpb9 to coordinate the opening/closing of the DNA Cleft and the binding of Switch 1 to the transcription fork of the RNA pol II, suggesting a role for Rpb5 in the transition from transcription initiation to elongation. Furthermore, this main function could therefore be extended to the RNA pol I and III enzymes, as well as to their counterparts in the archaeal and viral enzymes. In this work we show that Rpb5 has a specific role in the transcription elongation mediated by the RNA pol II. In fact the *rpb5-P151T* mutant affects transcription elongation, altering the processivity of the RNA pol II and the association of Spt5 elongation factor with the RNA pol II.

PS2-12: Cth2 acts as a translational regulator of ARE-containing transcripts under iron deficiency

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Iron (Fe) acts as an essential cofactor in a wide range of cellular processes. Although iron is very abundant, its low solubility at physiological pH compromises its bioavailability, and therefore cells have developed sophisticated mechanisms to control Fe homeostasis, both at the transcriptional and post-transcriptional level. In response to Fe deficiency, the yeast *Saccharomyces cerevisiae* induces the expression of Cth2, an RNA binding protein that promotes a remodeling of cellular metabolism directed to optimize iron utilization. Cth2 protein exerts its function through its two CCCH tandem zinc fingers (TZFs) that bind to AU-rich elements (AREs) within the 3' untranslated region (3' UTR) of specific mRNAs. Mature Cth2-mRNA complexes are exported to the cytosol, where Cth2 interacts with the helicase Dhh1 and promotes the degradation of its targets via the 5' to 3' decay machinery. Thus, Cth2-mediated mRNA decay provokes the downregulation of non-essential iron dependent processes, such as respiration, while essential ones such as DNA synthesis are upregulated. Cth2 targets include *CTH2* mRNA, in an auto-regulatory mechanism, and *SDH4*, which encodes a subunit of Succinate Dehydrogenase. Our previous results show that mutation of the AREs in these transcripts causes an increase in both mRNA and protein, which, not being correlated, suggests a translational regulation of these mRNAs. Additionally, Dhh1 is also known to act as a repressor of translation, while ARE elements have been involved in translational regulation in mammals, through interactions with proteins such as the Cth2 homolog Tristetraprolin (TTP), which represses translation in an RCK/p54/Dhh1 dependent manner. Altogether, these data have led us to investigate a possible role of Cth2 in translational regulation of *CTH2* and *SDH4* mRNAs. Our results support that Cth2 participates in ARE-mediated translation inhibition under iron deficient conditions.

PS2-13: Natural variation in yeast uncovers novel regulation of the Ena1p sodium pump
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All organisms must recognize and respond to various environmental stresses throughout their lifetime. Salt stress is commonly encountered in natural environments, and cells have developed strategic mechanisms to maintain low intracellular sodium levels during salt exposure in an attempt to survive. The budding yeast *Saccharomyces cerevisiae* has long been a model for understanding eukaryotic salt tolerance. In yeast, induction of the Ena1p sodium efflux pump reduces intracellular sodium levels when cells face life-threatening salt concentrations. The regulation of *ENA1* is surprisingly complex, coordinating multiple signal transduction cascades, transcription factors, and environmental cues. However, our understanding of *ENA1* regulation has been largely limited to commonly used laboratory strains. While investigating natural variation in the yeast ethanol response, we made a remarkable discovery – we found that transcription of *ENA1* was activated by ethanol in a wild vineyard isolate, but not in the laboratory strain. This connection between ethanol stress and salt stress then led to the identification of a new role for *ENA1* in salt cross-protection by ethanol, which is absent in the lab strain as well. To understand the genetic basis of these newfound regulations of *ENA1*, we are utilizing promoter swapping to construct hybrids of the lab strain and wild vineyard strain that differ only by whether they contain the lab or vineyard *ENA1* allele, and subsequently evaluate by quantitative real-time PCR (Q-PCR) and growth curve analysis. This design will allow us to test whether the expression differences between the lab and wild alleles of *ENA1* are due to local effects (e.g. promoter mutation) or distant effects (e.g. transcription factor mutation). By exploiting the natural variation between wild and laboratory yeast, we are providing new insights into the regulation of the well-studied Ena1p sodium pump system, and the molecular mechanisms that underlie gene expression variation.

PS2-14: Accelerated mRNA degradation contributes to gene expression remodeling during a nitrogen upshift**Darach Miller**, Benjamin Neymotin, David Gresham*Center for Genomics and Systems Biology, New York University, New York City, New York, USA*

Budding yeast uses conserved signaling pathways to integrate information about the environment into a growth rate decision. In cells growing in diverse nutrient limitations the expression of about a quarter of the yeast transcriptome is determined by growth-rate, largely independent of which type of nutrient is limited. We used nitrogen limitation in yeast as a system to study how a eukaryotic cell coordinates growth-rate dependent regulation of gene expression. A nitrogen upshift, from slow growth on limiting or non-preferred sources to rapid growth on abundant preferred nitrogen sources, triggers a large-scale remodeling of the yeast transcriptome. This includes a rapid activation of RP/RiBi regulons and the repression of the Nitrogen Catabolite Repression (NCR) regulon, genes important for the utilization of non-preferred nitrogen sources. Using comparative modeling of the dynamics of mRNA abundance during the upshift and steady-state measurements of mRNA stability (RATE-seq), we show that acceleration of mRNA degradation contributes to the rapid down-regulation of some NCR transcripts during the upshift, particularly nitrogen-source transporters such as GAP1 and DIP5. We also observe that a nitrogen upshift causes a transient halt in cell-cycle progression consistent with a model of PKA and CLN1 mediated cell-cycle halt. We describe complementary high-throughput genetics approaches to determine how conserved signaling pathways execute these regulatory strategies to resume rapid-growth during a nutrient upshift.

PS2-15: How does yeast regulate translation? SnoRNA processing in *S. cerevisiae***Anna M. Mleczko**, Tomasz Twardowski, Kamilla Bąkowska-Żywicka*Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland*

Small nucleolar RNAs (snoRNAs) are one of the most ancient and evolutionarily conserved non-protein coding RNAs. Their primary role - the same in Archaea and all eukaryotic lineages - is to guide chemical modifications of other RNAs, mainly ribosomal RNAs, transfer RNAs and small nuclear RNAs. These modifications are of two prevalent types, 2'-O-ribose methylation, guided by C/D family, or pseudouridylation, guided by H/ACA family of snoRNAs. In addition to this primary role, snoRNA can possess other functions. For example, stress conditions can induce cleavage of snoRNAs in specific sites and forming of stable processing products known as snoRNA-derived RNAs (sdRNAs). sdRNAs were found in multiple species, ranging from mammalian species to *Giardia lamblia* and Epstein-Barr virus. By deep-sequencing of a specialized ribosomal cDNA library we were able to select over 200 putative ribosome-associated ncRNAs - snoRNAs and sdRNAs among them. Interestingly, it has been shown that snoRNAs associate with components of the RNA interference pathway, and a human and a protozoan snoRNA can be processed into microRNA-like RNAs. Although the components of the machinery necessary for microRNA action are conserved in diverse eukaryotic species, it has been lost in the budding yeast *Saccharomyces cerevisiae* hence this organism is an ideal system for studying sdRNAs role independently of RNAi pathway. Thus the essential question we are asking is: Do small RNAs processed form snoRNA play a role in a regulation of gene expression in *Saccharomyces cerevisiae* in RNAi independent way? What is more, we also want to discover where in the cell and under which environmental conditions cleavage of full – length snoRNA occurs. Here, we present the first evidences that snoRNA fragments can regulate protein biosynthesis in eukaryotic organisms.

PS2-16: The RNA helicase Ded1 differentially controls translation of mRNAs with specific secondary structures in vivo**Diana S.M. Ottoz***Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*

Translation initiation in *Saccharomyces cerevisiae* is a highly regulated process involving initiation factors to assist the loading of the ribosome on the mRNA and the identification of the start codon. After binding to the capped 5' end, the small ribosomal subunit scans the sequence of the mRNA toward the 3' end, searching for the start codon. To allow the scanning of the 5'-untranslated region (5'-UTR) preceding the start codon, the RNA secondary structures need to be solved. Several RNA helicases are involved in translation initiation. Among

them, the essential Ded1 assists the small ribosomal subunit during scanning of long 5'-UTRs. Although the RNA unwinding activity of Ded1 has been well studied in vitro, the impact of this helicase during scanning in vivo is not fully characterized. Here I describe an in vivo assay to analyze the action of Ded1 on mRNAs containing specific secondary structures in their 5'-UTR. I titrated the expression of DED1 with an inducible heterologous transcription factor. I analyzed the effect of this perturbation on the translation efficiency of mRNAs containing specific structures in their 5'-UTR. I measured the expression levels of the mRNAs, which encoded a fluorescence protein, by flow cytometry. Ded1 titration increased the expression of mRNAs containing a long 5'-UTR, but not of mRNAs with a short 5'-UTR, confirming Ded1's specific role for long 5'-UTRs. I analyzed the effects of Ded1 titration on four long 5'-UTRs containing zero, one, or two stem loop structures in different positions. High amounts of Ded1 increased the translation of all four mRNAs. I observed a stronger increase of the expression when one stem loop was placed near the 5'-cap, compared to when it was placed next to the start codon. The strong impact of the stem loop near the 5'-cap was hardly affected when a second stem loop was placed next to the start codon. These results indicate a stronger impact of Ded1 on the 5' portion of the 5'-UTR. The mRNA without stem loop structures was the least affected by Ded1 titration. These results demonstrate that with this assay it is possible to characterize the impact of Ded1 on specific 5'-UTR features, like length and secondary structure. This contributes to a better understanding of the role of Ded1 in the scanning mechanism and of its importance in the regulation of translation initiation.

PS2-17: Control of gene expression by the yeast degradation factors Xrn1 and Dhh1 during the response to hyperosmotic stress

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Recent studies strongly suggest that regulation of gene transcription at the nucleus is mechanistically connected, not only with nuclear mRNA processing, but also with cytoplasmic mRNA decay. The crosstalk between degradation and synthesis would serve to maintain proper mRNA levels in the cell. In this context, factors of the mRNA decay machinery, such as the 5'-3' exonuclease Xrn1, have been found to bind chromatin and, under steady-state conditions, depletion of Xrn1 decreases mRNA synthesis and increases mRNA half-life in a compensatory manner. Here, we investigate the role of these crosstalk factors under stress conditions in which mRNA levels are rapidly and profoundly changed in a transitory manner. Mutants in Xrn1 and in the decapping activator factor Dhh1 show resistance to stress caused by high osmolarity. Indeed, deletion of *xrn1* and *dhh1* results in accumulation of osmo-mRNAs during the osmotic stress response. Interestingly, we observed high levels of RNA polymerase binding to osmo-gene promoters in *xrn1* and *dhh1* mutants, however, a genome-wide study of transcription rates showed that RNA polymerase II transcription is deficient in *xrn1*, therefore confirming a role of Xrn1 in transcription elongation during stress. Altogether, our results indicate that Xrn1 is necessary for transcription and degradation of osmo-mRNAs in response to osmotic stress, however the altered osmo-mRNA kinetics observed in *xrn1* and *dhh1* suggest that the lack of these decay factors causes a perturbation in the fine-tuning of the mRNA synthesis-degradation crosstalk during osmotic stress, which could be beneficial for the yeast cells.

PS2-18: RNA polymerase III activity affects glucose transport in *S.cerevisiae*.

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RNA Polymerase III (RNAP III) activity is regulated by a general repressor Maf1 protein. Maf1 deficiency affects *maf1-Δ* strains growth on non-fermentable carbon source at elevated temperature and influences cellular processes such as tRNA accumulation [1, 2]. The phenotypic effects of MAF1 deletion is suppressed by a Gly1007Ala point mutation in the second largest RNAP III subunit – C128 [3]. Microarrays studies have contributed to the understanding of gene expression patterns in *maf1-Δ* during cultivation on non-fermentable carbon source. We observed several genes transcribed by RNA Polymerase II (RNAP II) have changed

expression profile, among others, HXT genes encoding high-affinity glucose transporters. Real-Time qPCR was employed to verify the ‘omics data. Glucose transporter transcript levels are remarkably different in both *maf1*-Δ and *rpc128-1007* grown on glucose and glycerol-based medium, in comparison to wild type strain. Label free proteomics data suggest that *rpc128-1007* and *maf1*-Δ mutants represent diverse life strategies in nutrient uptake and metabolism under the same growth conditions. This involves differences in protein abundance of enzymes engaged in amino acids synthesis and catabolism as well as glucose metabolism. The ‘omics data correlate with enzyme activity measured by *in vitro* assay [4]. Since glucose metabolism is inextricably tied to glucose uptake, the glucose transport efficiency has been examined and presented. The results suggest deregulation of the glucose signaling network in the mutants.

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PS2-19: The Yeast SAGA Complex Cooperates with SWI/SNF to Regulate Gene Expression though the Cell Wall Integrity MAPK Pathway.

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In *Saccharomyces cerevisiae*, the transcriptional program triggered by cell wall stress is coordinated by Slt2, the MAPK of the CWI pathway, mainly through the transcription factor Rlm1. To identify novel elements required for proper gene expression under conditions affecting cellular integrity, we performed a large-scale screening to isolate mutant cells defective in the induction of the CWI transcriptional response. Several protein complexes related to transcription were identified, including the SWI/SNF ATP-dependent chromatin remodeling and SAGA histone-modifying complexes. Rlm1 interacts with SWI/SNF complex to direct its association with target promoters. Once recruited, SWI/SNF locally alters nucleosome positioning, allowing Rlm1 binding at the previously occluded Rlm1 binding site and the accessibility of the RNA Poll II to DNA (1). However, transcription initiation is a complicated process that requires the coordinated activities of not only one co-activator but a large number of factors to ensure an appropriate regulation upon stress. In this context, here we show that the SAGA complex plays along with the chromatin remodeling SWI/SNF complex a critical role in orchestrating the transcriptional responses regulated by Rlm1 in response to cell wall stress. HAT saga mutants display hypersensitivity to the cell wall interfering compound Congo red and genome-wide transcriptional analysis revealed that Gcn5 co-regulates together with Swi3 the majority of the transcriptional response induced upon stress. Furthermore, the SAGA complex enters on cell wall responsive gene promoters under cell wall stress to mediate H3 acetylation, cooperating with the SWI/SNF complex for eliciting nucleosome reorganization and gene expression through the CWI pathway.

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PS2-20: Hypoxia and glucose regulate transcription of the low-affinity glucose transporter gene *RAG1* in *Kluyveromyces lactis*

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Glucose signaling and glycolysis regulates the expression of the low-affinity glucose transporter gene *RAG1* in *Kluyveromyces lactis*. Glucose signaling acts through cascades involving the glucose sensor Rag4 and downstream proteins. A major role is exerted by the casein kinase Rag8 and the repressor proteins Sms1 and KIRgt1. Another pathway involves the chromatin remodeler KISnf2 and Sck1 and also signals from glycolysis are involved. We have found that transcription of *RAG1* is also induced by hypoxia and this induction requires the presence of glucose. Molecular dissection and structural analysis of the *RAG1* promoter allowed to identify the region essential for the induction. Transcription analysis of *RAG1* in various mutant strains of the glucose

regulation allowed to identify Sck1 as the possible element of connection between glucose and oxygen signaling. Dependence of Sck1 expression on hypoxia and binding of Sck1 to the *RAG1* promoter has been also investigated. Interestingly, the level of *RAG1* transcription, but not the hypoxic induction, depended on the presence of the hypoxic transcription regulator KIMga2. Our results show that the expression of the glucose transporter gene *RAG1* is synergistically regulated by high glucose and low oxygen and the glucose regulator Sck1 and the hypoxic regulator KIMga2 might cooperate in this mechanism. NuSA analysis of the promoter in the pertinent physiological conditions and mutant strains, indicates the role of chromatin organization in *RAG1* transcriptional regulation. This work was partially funded by Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese

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PS2-21: A shared regulatory network allows functional coupling of Pho89 and Ena1 in response to environmental alkalization

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Exposure to alkaline pH triggers a stress response in *S. cerevisiae* which results in the activation of a complex regulatory network composed of a multitude of different signaling pathways. This leads to a profound remodeling of the transcriptional profile and the expression of hundreds of genes. We show that the expression under alkaline conditions of the high-affinity Na⁺/Pi cotransporter Pho89 depends on at least four different pathways. The phosphate-responsive *PHO* pathway, also activated by high pH stress, induces the expression of the *PHO* regulon, including Pho89. Additionally, this transporter is upregulated by the calcineurin pathway, which responds to the cytosolic calcium burst occurring after alkaline stress. Moreover, Pho89 is also regulated by the repressors Nrg1/Nrg2 and Mig2. The activation of the Rim101 pathway under alkaline conditions leads to the downregulation of *NRG1*, thus relieving the repression of this transcription factor on *PHO89*. We observe that high pH stress causes a fast but transient phosphorylation of the Snf1 kinase, and that Mig1 and Mig2 are also phosphorylated following the same timing in a Snf1-dependent manner. Furthermore, their phosphorylated state is coincident with their nuclear exclusion after the alkaline stress. Interestingly, we observed that the *reg1* strain, which presents a hyperactive Snf1, exhibits a drastic increase in Pho89 alkaline induction, especially at a long-term. Our results suggest that, apart from impinging on Pho89 through the Mig2 repressor, Snf1 and Reg1 might be regulating the transporter stability, as previously described for other plasma membrane proteins. The network controlling the induction of Pho89 under alkaline conditions reported in this work is essentially identical to that described for the Na⁺-ATPase-encoding gene *ENA1*. We present data showing that these similarities lead to their synchronous expression after high pH stress. Moreover, we observe that the increased Pho89 activity under an alkaline environment results in a detrimental accumulation of intracellular sodium, and that in this situation the sodium extrusion activity of Ena1 serves as a detoxifying mechanism. Therefore, the common regulatory network of Pho89 and Ena1 is at the basis of their functional coupling to allow cells to thrive in such adverse environment.

PS2-22: Structure-function studies of the ELL-EAF complex of *Schizosaccharomyces pombe*

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Transcription of protein-coding genes requires the coordinated action of different proteins, and is an important step in the control of gene expression in eukaryotic cells. Research over the past few decades has primarily focussed on the preinitiation and initiation stages of transcription. However, several recent studies have shown that transcription elongation also constitutes a major step of transcription regulation, wherein transcription elongation factors play a significant role. A plethora of these elongation proteins have been discovered, and

elucidating the roles of each of these proteins is a challenge. The ELL (Eleven Nineteen Lysine Rich Leukemia) and EAF (ELL associated factor) family of proteins have been identified in *in vitro* transcription assays to suppress transient pausing of the RNA polymerase II enzyme along the DNA template. A single homologue of ELL and EAF proteins is present in *Schizosaccharomyces pombe*. In this study, we have used different approaches in an attempt to characterize the molecular, functional and structural properties of these proteins. Our results show that deletion of either ELL or EAF results in slow growth of cells under optimum growth conditions. Moreover, exposure to DNA damaging agents reduces the viability of ELL and EAF null mutants. In comparison, no defect in growth of these cells was observed under a variety of environmental stress conditions tested in the study. To further delineate the regions of these proteins that are important for the observed phenotypes, we have constructed a series of ELL and EAF truncation mutants, and tested them for their ability to rescue these phenotypes. Our deletion mutant analysis provides evidence that the carboxyl terminal region of the EAF protein plays an important role in the survival of cells under DNA damaging conditions. Using reporter gene activation assays, we show that this region also has transactivation potential. We also observed that the human ELL and EAF proteins could not complement the absence of these proteins in *S. pombe*, and in agreement with these observations, our yeast two hybrid analysis showed no interaction between the *S. pombe* and human ELL-EAF proteins. We have also used computational and biophysical approaches to gain structural insights into these proteins. In summary, our results provide insights into the function(s) and structure of these proteins in *S. pombe*.

PS2-23: Dealing with spontaneous wine fermentation: wild yeast behaviour

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High sugar fermentations present a stressful environment for yeast due to very high initial osmotic pressure and consequently high concentrations of ethanol as a product of alcoholic fermentation. This translates to more likelihood of sluggish and stuck fermentations. "Spontaneous" fermentations in which the natural micro-flora present on the grapes/in the winery are responsible for the alcoholic fermentation are favoured for their added complexity even though they can be less tolerant of conditions in juices of even average sugar content. The research into the behaviour of non-*Saccharomyces* yeast is not as extensive as that for *Saccharomyces*. In particular the molecular basis of oenological properties of these so-called wild yeast needs to be defined under the range of conditions and oenological practice seen between wineries. An in-house collection of wild yeast isolates has been created and the identification of non-*Saccharomyces* and *Saccharomyces* species undertaken. The fitness of these yeast species was measured individually in environments mimicking increasing stresses, especially osmotic and ethanol stress, found in the wine fermentation. Differences in growth rate and fermentative metabolism were defined. Supplements, known to be stress protectants, were used to test their ability to enhance yeast survival and sugar consumption. Species-specific PCR primers were used to study the effects of stress in a mixed population, along with the fermentation kinetics and wine chemical profile. Understanding what metabolic changes that yeast cells undergo in order to deal with high sugar environments will be the focus in the subsequent stage of this study. Genetic expression and fermentation behaviour are going to be monitored to determine side effects of different genetic expression patterns on functionality and final products. This information will give winemakers guidelines on how to avoid risky fermentations and also how to modulate yeast growth and sensory contribution.

PS2-24: Transcriptional response of *Saccharomyces cerevisiae* to potassium starvation

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Ion homeostasis is essential for every cell and aberrant cation homeostasis is related to diseases like Alzheimer's disease and epilepsy. Intracellular potassium concentrations are kept relatively high, whereas sodium concentrations are in general more than tenfold lower. The mechanisms responsible for cation homeostasis are only partly understood. The yeast *Saccharomyces cerevisiae* is an excellent organism to study fundamental aspects of cation homeostasis. We investigated the transcriptional response of this yeast to potassium starvation by using SAGE-tag sequencing. Comparison of transcript levels in cells grown for 60 min in media without potassium with those in cells grown under standard potassium concentrations showed that the mRNA levels of

105 genes were significantly ($P<0.01$) up-regulated more than 2.0-fold during potassium starvation and the mRNA levels of 172 genes significantly down-regulated. Up-regulated genes belong to several functional categories, including stress response and phosphate metabolism. Analysis of selected promoters revealed that increased RNA levels are caused by both increased transcription and decreased RNA turnover. In the latter process antisense transcription may be involved. Our study showed that yeast cells respond to potassium starvation in a complex way and that potassium starvation activates genes involved in phosphate metabolism like *PHO84* and *SPL2*.

[1] Anemaet IG and van Heusden GPH (2014) *BMC Genomics* 15, 1040.

PS2-25: Aneuploidy affects the nonsense suppression efficiency in the *Saccharomyces cerevisiae* strains with impaired function of the translation termination factor eRF3

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The SUP35 gene of the yeast *Saccharomyces cerevisiae* encodes the translation termination factor eRF3. Mutations in this gene lead to the suppression of nonsense mutations and a number of other pleiotropic phenotypes, one of which is impaired chromosome segregation during cell division. Similar effects result from replacing the *S. cerevisiae* SUP35 gene with its orthologues. Different genetic and epigenetic changes occurring in the sup35 background result in partial compensation for this suppressor effect. We previously found a novel antisuppressor determinant Asp+ in *S. cerevisiae* strains in which the SUP35 orthologue from the yeast *Pichia methanolica* replaced the *S. cerevisiae* SUP35 gene. Now we have shown that antisuppressor effect of Asp+ is determined by disomy of the chromosome VIII. This antisuppressor effect is not associated with decreased stop codon readthrough, as follows from the results of dual-luciferase assay. We identified SBP1, a gene that localises to chromosome VIII, as a dosage-dependent antisuppressor that strongly contributes to the overall antisuppressor effect of chromosome VIII disomy. The SBP1 gene codes for an RNA-binding protein that functions in mRNA degradation and translational repression. Overproduced Sbp1p may explore its antisuppressor effect through influencing the stability, degradation, and / or efficiency of the translation of the transcripts of the genes containing marker nonsense mutations. We observed also multiple pleiotropic effects of VIII chromosome disomy in our strains: increased tolerance of the copper salts, hypersensitivity to the salts of different transition metals including zinc, manganese, iron, cobalt, nickel, and cadmium, hypersensitivity to oxidative stress, change in the vacuole structure and/or biogenesis, etc. We tried to study effect of disomy of other chromosomes on the nonsense suppression efficiency using “exclusive” cytoduction with transfer of single chromosomes from the donor to the recipient. We have shown that disomy of the chromosome XVI also leads to antisuppressor effect. Thus, appearance of aneuploidy of at least chromosomes VIII and XVI may be one of the mechanisms of modulation of nonsense suppression in yeast strains with impaired translation termination. The effects of aneuploidy of other chromosomes on the nonsense suppression efficiency are under investigation. Supported with St-Petersburg University research grants 0.37.696.2013 and 1.37.291.2015, RFBR grant 15-04-08159.

Poster Session 3: Growth control and metabolism**PS3-1: Maf1 protein Has a wide effect on metabolism in *Saccharomyces cerevisiae***

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Cancer cells physiology and metabolism are very similar to rapidly proliferating yeast. Enzymes involved in carbohydrates and lipid metabolism have been found to play a major role in cancer cell proliferation and most of these enzymes are conserved in the yeast, *Saccharomyces cerevisiae*. Both types of cells display highly active glycolysis and pathways for synthesis of fatty acids. Maf1 is a negative regulator of RNA polymerase III in yeast and higher eukaryotes during growth-limiting conditions. *maf1*-Δ cells were observed to elevate tRNA transcription and lack of growing on non-fermentable carbon source [1, 2]. Additionally Δ*maf1* mutant marks lower transcription level for genes involved in cytoplasmic fatty acids biosynthesis pathway FAS I. However counter-intuitively, the accumulation of oleic acid is observed in this strain. To elucidate observations we use the genome-scale mathematical model for simulating *maf1*-Δ mutant metabolism. Application of Surrey Flux Balance Analysis employing iTO977 model has shown an alternative metabolic flux through mitochondrial Fatty Acids Synthesis Type II pathway (FAS II) [3, 4]. FAS II pathway is independent from the cytosolic FAS I pathway. The data generated *in silico* have been verified experimentally.

[1] M. Cieśla et al (2007) *Molecular and Cellular Biology*, Vol. 27, 7693-702 ; [2] E. Morawiec et al (2013) *Gene*, 526, 16-22; [3]. A. Gevorgyan et al (2010) *Bioinformatics*, 27,433-434; [4]. Österlund T. et al (2013) *BMC Systems Biology*, 7, 36.

PS3-2: The protein phosphatase Ppz1 as a potential antifungal drug target: Deciphering the molecular basis of its function and regulation

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Invasive fungal infections constitute an enormous threat, particularly in immunocompromised individuals, and the increasing incidence of fungal infections has aggravated the need for novel approaches to antifungal therapies. Years ago our laboratory discovered in *S. cerevisiae* the protein phosphatase (PPase) Ppz1, which plays a key role in monovalent cation (K^+ and Na^+) homeostasis and, hence, in multiple cellular processes. Ppz-like enzymes are only found in fungi, including pathogenic ones, such as *C. albicans* or *A. fumigatus*. In *S. cerevisiae*, Ppz1 activity is inhibited in vivo by two similar regulatory subunits, Hal3 and Vhs3, which are actually moonlighting proteins, also involved in CoA biosynthesis. We reported long time ago that high levels of Ppz1 are deleterious for the cell, and a recently genome-wide approach has demonstrated that, when overexpressed in high-copy number, Ppz1 is the most toxic protein in budding yeast. Interestingly, current evidence suggest that the way Hal3 interacts and inhibits Ppz1 differs from the regulatory mechanism of the widespread eukaryotic type 1 protein phosphatase. Our laboratory aims to understand the basis of Ppz1 function and regulation as a way to evaluate the potential role of Ppz1 as a target in antifungal therapy. We will present progress being made in several fronts. 1) Genetic approaches to identify residues in Ppz1 necessary for interaction with Hal3 or crucial for its inhibition. 2) the identification of the molecular basis for Ppz1 toxicity, 3) evaluation of *in vivo* phosphorylation of Ppz1 and Hal3 as possible regulatory mechanisms, and 4) assessment of the biological effect of hyperactivation of Ppz-homologs in pathogenic fungi. These results may lead to the recognition of Ppz1 as a novel drug target and assist in developing drugs that, by interfering with the inhibitory role of Hal3, would result in toxicity for pathogenic fungal cells.

PS3-3: Physiological impact of NAD(P)HX accumulation in an NADHX repair-deficient yeast model

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One of the revelations of the genomics revolution is that for most organisms a majority of encoded proteins remains without identified function. Many of those unknown proteins are members of widely conserved families and a significant proportion of them are probably enzymes. Accumulating evidence suggests that many enzymes of unknown function are involved in metabolite damage control, an aspect of metabolism that has been largely neglected so far. Abnormal metabolites are constantly generated inside the cell by unwanted chemical reactions or by enzymatic side reactions; they are useless at best and toxic at worst. Metabolite repair enzymes clear the metabolite pool of these non-canonical metabolites. Their importance is well illustrated through implication in disease processes. L-2-hydroxyglutaric aciduria for example, a severe human neurometabolic disorder, is caused by a deficiency in a metabolite repair enzyme. The latter normally prevents accumulation of a toxic side product formed by L-malate dehydrogenase. A series of other metabolite repair enzymes have been identified in mammals, but also in plants, invertebrates and microbes, including yeast. Among these, two enzymes that specifically repair NAD(P)HX, hydrated and therefore inactive forms of NAD(P)H, have recently been biochemically characterized: an NAD(P)HX dehydratase and an NAD(P)HX epimerase, encoded by the yeast YKL151C and YNL200C genes, respectively. The physiological relevance of these two enzymes still remains largely unclear. To address this question, yeast knockout strains lacking the NAD(P)HX repair enzymes were generated in auxotrophic and prototrophic backgrounds. By systematic analysis of cell extracts of these strains using HPLC and LC-MS methods enabled us to demonstrate that NADHX and NADPHX can be formed intracellularly. Higher levels of NADHX were found in NADHX repair deficient strains when cells were grown at higher temperatures. Overexpression of 2 yeast isoforms of the glycolytic enzyme GAPDH also led to an increase in intracellular NADHX formation. Along with the accumulation of NADHX, a decrease in the intracellular NAD⁺ level was observed in NADHX repair deficient strains. Finally, preliminary metabolomics analyses and RNA tiling arrays suggest that NAD(P)HX repair deficiency leads to changes in amino acid metabolism as well as in histone expression.

PS3-4: Characterization of Kif4A as a novel mitotic iron-sulfur protein

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Chromosome Instability (CIN) is a preliminary condition for the development of cancer. Recent studies have attempted to expand the list of genes contributing to the maintenance of chromosome stability in yeast. These studies resulted in the identification of many yeast chromosome instability (CIN) genes which can be organized into a number of functional groups. One of the identified biological pathways is the biosynthesis of iron-sulfur (Fe-S) proteins. The maturation of the cytosolic iron-sulfur proteins is carried out by the cytoplasmic iron-sulfur protein assembly (CIA) machinery. Our results provide strong evidence for the co-localization of the CIA complex with elements of the mitotic machinery. Moreover, knockdown of Mms19 and hCia2 lead to a poor alignment of chromosomes to the metaphase plate and multi polar spindle pole bodies during mitosis. In this work we propose that Kif4A, chromokinesin that localizes to midzone and midbody during cytokinesis is one of the potential targets of the CIA complex. We show that CIA2, one of the CIA complex members, and Kif4a are co-localize to the midbody in mitotic cells. Moreover, we found that cystein rich domain(CRD) which kif4a harbors at its C-terminus is capable of incorporating 55Fe by using biochemical assays. Furthermore, CRD is important for the proper localization of Kif4A to the midbody and for its interaction with CIA complex member Mms19. Taking together, these results characterize kif4a as a novel mitotic iron-sulfur protein involved in chromosome segregation.

PS3-5: The role of Clc1p and Chc1p in endocytosis/exocytosis in yeast**Bayram Cucu**, Lucia Carillo, Daniel Degreif, Max Kohl, Gerhard Thiel, Adam Bertl*Yeast Membrane Biology, Department of Biology, Technische Universität Darmstadt, Darmstadt, Hessen, Germany*

Clathrin-mediated endocytosis is the major pathway by which yeast cells internalize portions of the plasma membrane. This multi-step process requires the coordinated activity of a series of proteins, including Clc1p and Chc1p which form a protein coat that encloses the budding vesicle prior to internalization. Yeast strains deleted in either of the genes encoding clathrin chains (*CLC1*, *CHC1*) showed similar phenotypes, including slow growth and abnormal cell morphologies. Cells were spherical and significantly larger than WT. Experiments with yeast protoplasts, however, revealed different roles for Clc1p and Chc1p in endocytosis/exocytosis. In contrast to the *chc1* deletion, protoplasts from the *clc1* deletion strain showed significantly faster growth (volume increase) compared to WT. High-resolution membrane capacitance measurements showed that the faster volume increase of the *clc1Δ* protoplasts was the result of a significant imbalance between exocytosis and endocytosis, brought about by a strong reduction of the frequency of endocytic events, rather than by an increase in exocytotic events. No change in frequency of exocytotic events or size of endo-/exocytotic vesicles was observed. Deletion of CHC1 had no influence on the vesicle sizes or on the frequency of endo-/exocytotic events. The data from the electrophysiological experiments will be compared with the kinetics of incorporation/internalisation of fluorescently labelled membrane proteins into and from the plasma membrane.

PS3-6: Temporal localization characterization of the Fe-S clusters insertion into specific apo-proteins that participate in genome stability maintenance**Galit David-Kadoch**, Shay Ben-Aroya*Bar Ilan University, Israel*

Chromosome Instability (CIN) occurs at a low rate in normal cells but at a higher rate in cancer cells and is known as 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. This screen identified that proteins involved in mitochondrial and cytosolic Fe-S cluster biogenesis represented a major functional group. Fe-S clusters are small inorganic cofactors found in hundreds of proteins and are required in virtually all organisms from bacteria to humans. Mitochondria plays a primary role in the biosynthesis of Fe-S clusters in eukaryotes, as they are involved in the maturation of all cellular Fe-S proteins including cytoplasmic and nuclear ones. Biogenesis of the latter proteins additionally requires the cytoplasmic iron-sulfur protein assembly (CIA) machinery. Recent studies have shown that the CIA-machinery targets Fe-S clusters to several apoproteins involved in DNA-metabolism such as DNA-repair enzymes and DNA-polymerases. The majority of these target proteins function inside the nucleus, while the CIA complex is defined as a cytosolic complex. It is however unknown as to where the interactions between the CIA complex members and the target proteins occur. To elucidate this mechanism, we use the well-characterized Fe-S yeast protein Rad3, an essential DNA-helicase involved in nucleotide excision repair (NER) and transcription, in the context of the “Anchor-away-technique”. The anchor-away technique can be used to deplete a gene product of interest (the target) by conditional tethering to a suitable receptor protein (the anchor) located either in the cytoplasm or the nucleus. The rationale behind the anchor-away technique is the ability to trap the Fe-S protein of interest on the chromatin, thereby inhibiting its cytosolic localization. We can then conduct co-IP experiments to test if the permanent nuclear localization impairs the interaction of the tested protein with members of the CIA complex. Furthermore, an *in vivo* radiolabeling-immunoprecipitation assay can be used to directly test the levels of the modification with the Fe-S cluster. The function of Fe-S clusters in the maturation of crucial components of DNA metabolism is an important mechanism. Elucidating the molecular basis will help understanding the final crucial step of targeting Fe-S clusters to apoproteins involved in genomic stability.

PS3-7:A novel flocculation pathway mediated by ER membrane fluidity

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Appropriate flocculation characteristics of budding yeast (*S. cerevisiae*) allow sedimentation to be used in industrial and biotechnological applications as a simple, effective and low-cost method for separating cells from the fermentation broth. Flocculation in yeast is mediated by *FLO* gene encoded proteins, a family of cell wall anchored glycoproteins that can bind polysaccharides in the cell wall of adjacent cells. In wild yeast environmental stress conditions such as hypoxia or nutritional deficiencies trigger the expression of these proteins, also referred to as adhesins or flocculins. To investigate physiological effects of altered membrane lipid composition and modulated physicochemical membrane characteristics we have generated a yeast strain in which the extent of phospholipid unsaturation can be modulated by titrating the expression of *OLE1*. Ole1p is the sole fatty acid desaturase found in budding yeast and is consequently a key player in the regulatory network for maintaining physiological membrane fluidity. We found that reduced levels of unsaturation in membrane phospholipid incorporated fatty acids resulted in a strong flocculation phenotype. This was surprising since the laboratory strain used in this study is regarded as being non-flocculant due to a nonsense mutation in a gene that encodes for the canonical flocculation transcription activator. The identified novel pathway for induction of flocculation in *S. cerevisiae* involves instead the ER membrane resident fluidity sensors Spt23p and Mga2p whose activated forms directly drive the transcription of *FLO1*. Ole1p catalyzed desaturation of fatty acids requires molecular oxygen which consequently causes strong sensitivity of lipid desaturase activity to drops in dissolved oxygen concentrations. We hypothesize that the novel pathway presented here is responsible for hypoxia-induced flocculation long observed in brewing yeast strains. The insights from this study can advance the understanding of induction of flocculation in fermentation processes and may be exploited to optimize the flocculation behaviour of budding yeast strains used in industrial applications.

PS3-8: Intracellular acidification upon glucose depletion is regulated by PKA and TORC1 activity during growth

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In budding yeast, Protein Kinase A (PKA) and Target of Rapamycin (TOR) control growth in response to the nutrients carbon and nitrogen, respectively. In favorable conditions they promote growth, while upon stress or nutrient depletion they inhibit growth and trigger the activation of stress response mechanisms. Previous data from our group indicate that cytosolic pH (pH_c) also is a crucial regulator of growth. However, the mechanism for this control is unclear. Since pH_c responds to glucose availability we decided to investigate how pH_c and PKA collaborate in growth control. In order to test whether PKA pathway regulates pH_c, we analyzed pH_c during growth in a set of PKA mutants. We did not observe strong effects on pH_c during growth. However, upon glucose depletion, PKA over-activating mutants showed a stronger intracellular acidification, while low PKA activity mutants presented a reduced intracellular acidification. Similarly, in well controlled starvation experiments we observed that increasing PKA activity either by mutations or by addition of cAMP caused a stronger acidification upon glucose depletion, whereas inhibition of the kinase using a strain expressing an ATP-analog sensitive kinase subunit (*TPK1as*) delayed and reduced such acidification. Interestingly, these pH_c alterations upon glucose depletion were dependent on PKA activity set *before* starvation. PKA and TORC1 interact in order to ensure a more controlled regulation of their targets. We tested whether TOR also affected on pH_c after glucose starvation. Reduction of TORC1 activity either by nitrogen depletion or rapamycin addition reduced intracellular acidification. Our data indicate that TOR and PKA pathways together control pH_c upon glucose starvation. We are currently assessing the consequences of this pH_c-regulation on stationary phase-related phenotypes such as survival and stress response.

PS3-9: Yeast polyphosphatases as tools for polyphosphate assay

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Inorganic polyphosphate (PolyP), the linear polymer containing a few to several hundred phosphate residues, performs many functions in living cells. PolyP participates in the regulation of gene expression, stress response and virulence in bacterial cells. In human organism, PolyP is involved in the regulation of Ca²⁺ uptake in mitochondria, bone tissue development, blood coagulation, and brain functions. PolyPs are widely used as food additives. In view of the great role of PolyP in human organism, it is necessary to control its amounts in food. Enzymatic methods using exopolyphosphatases is prospective for PolyP assay. *S. cerevisiae* strains overexpressing *PPX1* and *PPN1* genes encoding yeast polyphosphatases under the control GAPDH promoter were constructed [1-2]. The recombinant enzymes were purified and their physico-chemical properties characterized. Both enzymes exhibit exopolyphosphatase activities, that split P_i from the end of the PolyP chain. PPX1 and PPN1 differ in the substrate specificity (Table).

Table. Substrate specificity of recombinant PPX1 and PPN1 of *S. cerevisiae* with PolyP_n where n is the average length of polyphosphate chain.

Substrate	Activity, %	
	PPX1	PPN1
Pyrophosphate	0	0
Tripolyphosphate	185	9
PolyP ₁₅	120	88
PolyP ₄₅	100	100
PolyP ₂₀₈	100	100

The PPX1 enzyme is active as a exopolyphosphatase in presence of Co²⁺ or Mg²⁺ and possesses no endopolyphosphatase activity. PPN1 is active as a exopolyphosphatase only in the presence of Co²⁺, and in the presence of Mg²⁺ it demonstrated an endopolyphosphatase activity, splitting the long-chained PolyP into shorter ones. Both enzymes have neutral pH optima, and are not inhibited by phosphate. The enzyme preparations preserve their activities for several month under -20° C in the presence of 0.1% triton X-100. The use of two enzyme preparations allows to assay tripolyphosphate and long-chain polyphosphates in food and biological samples in the presence of pyrophosphate and orthophosphate.

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PS3-10: The PWWP-containing protein Pdp3 regulates energetic metabolism in *Saccharomyces cerevisiae*

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WHSC1L1/NSD3, one of the most aggressive oncogenes from the 8p11-12 region, has two isoforms that are derived from alternative splicing of exon 10. Both isoforms contain a PWWP domain, which is a methyl-lysine recognition motif involved in histone code modification and epigenetic regulation of gene expression. Overexpression of long or short NSD3 isoform is capable of transforming a healthy into a cancer cell. Due to genetic and metabolic similarities between *Saccharomyces cerevisiae* and cancer cells, this microorganism has often been used as a tool for cancer research. We have identified the yeast protein PDP3 that closely resembles NSD3 and also contains a PWWP domain. Our goal was to elucidate the function of the PDP3 PWWP by analyzing the effects of its deficiency in yeast cells proliferation, switch from oxidative to fermentative metabolism, and tolerance to oxidative stress. We also investigated the effect of the overexpression of PDP3, short isoform of NSD3 (NSD3s), or a chimeric form of PDP3 carrying the NSD3 PWWP domain on those

parameters. Cells were grown in two different carbon sources, glucose or glycerol, until first exponential phase. A Clark electrode measured oxygen consumption. Tolerance against oxidative stress was analyzed before and after exposure to hydrogen peroxide by determining viability and lipid peroxidation. We observed that cells deficient in PDP3 or expressing the *PDP3* PWWP W21A mutant exhibited a decrease in specific growth rate (μ), a reduction in glucose consumption and a high tolerance to peroxide stress during growth on glucose, which favors fermentation. On the other hand, yeast cells overexpressing NSD3s or PDP3 behaved like tumorigenic cells under conditions that stimulate respiration (growth on glycerol): increased μ and decreased tolerance to oxidative stress. Our results indicate that the yeast protein PDP3 and the human NSD3s play similar roles in cell metabolism. The swapping domain experiments suggested that the PWWP domain of NSD3s functionally substitutes that of the yeast protein PDP3, whose hydrophobic cage is essential for its metabolic function.

PS3-11: Identification of novel genes related to partial PtdIns(4,5)P₂ elimination in *Saccharomyces cerevisiae* by mammalian PI3K heterologous expression

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Heterologous expression of mammalian class I PI3K catalytic subunit p110 α , a prominent target in cancer disease, in the model organism *Saccharomyces cerevisiae* leads to growth inhibition due to the conversion of the essential pool of PtdIns(4,5)P₂ from the plasma membrane into PtdIns(3,4,5)P₃. Whereas the latter is naturally absent in this organism, studies performed up to date with a thermosensitive allele of the PtdIns-4P-5-kinase MsS4, in which PtdIns(4,5)P₂ synthesis is abolished, have shown that this molecule is a key second messenger that regulates endocytosis, polarized exocytosis, actin polymerization and signaling in yeast. PI3K-expressing yeasts have proved to be a useful and alternative system to study the roles of PtdIns(4,5)P₂ in yeast biology, revealing, for instance, the influence of this lipid on cell wall integrity MAPK pathway and intracellular trafficking. To gain insight into PtdIns(4,5)P₂-regulated cellular processes, we have performed a visual screen on the whole non-essential gene deletion collection by expressing a myristoylated-PI3K version (Myr-p110 α) that leads to an intermediate growth inhibition when expressed on wild type yeast cells. This allowed us to detect mutants in which PI3K-dependent growth inhibition, led to an enhanced or attenuated phenotype. The screening was carried out by transforming the collection (4847 haploid strains) with a Myr-p110 α expression plasmid, achieving a transformation efficiency of 89%. After individual verification of primary hits in triplicate growth assays on agar media, we isolated 28 phenotypic suppressors of Myr-p110 α -induced toxicity and 22 phenotypic enhancers (0.6% and 0.5% from the total number of strains screened, respectively). Clustering of hits into functional groups revealed a statistically significant enrichment of genes related to mRNA processing among the suppressors of Myr-p110 α -dependent growth inhibition. Among the phenotypic enhancers, we found genes related to protein translation and Golgi apparatus. These results open a path to explore putative new roles of PtdIns(4,5)P₂ in the regulation of yeast basic cellular processes.

PS3-12: Can less energy mean more energy? Increased sugar to ethanol conversion in *Saccharomyces cerevisiae* via overexpression of ATPases

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Ethanol is an important biofuel produced in large scale and used in many countries as a substitute or as an additive to gasoline. It is mainly obtained by fermentation of sugar-containing raw materials, with the yeast *Saccharomyces cerevisiae*. In this industrial process, ethanol yield on sugar ($Y_{ETH/S}$) needs to be maximized, since raw material costs play a major role in the overall process economics. It has already been demonstrated that it is possible to increase $Y_{ETH/S}$ by decreasing free-energy conservation in *S. cerevisiae*, because under these conditions, cells need to increase the carbon flux towards ethanol formation, which is intrinsically coupled to

ATP generation when alcoholic fermentation is the only energy-conserving pathway available. Here we show that by overexpressing ATPases, *S. cerevisiae* is able to increase $Y_{ETH/S}$. For this purpose, we inserted integrative plasmids containing one of three native promoters (weak: CYC1, intermediate: ADH1 or strong: TEF1), followed by one of two native ATPase encoding genes HSP104 or CDC48, in *S. cerevisiae* CEN.PK113-5D. Cultivations using a defined medium with 5 g/L initial glucose at 30 °C and 150 min⁻¹ were performed under quasi-anaerobic conditions. The results showed a clear trend of increase in $Y_{ETH/S}$, when promoter strength was increased. Strains overexpressing the CDC48 ATPase gene presented higher $Y_{ETH/S}$ values than strains overexpressing the HSP104 ATPase. We also observed a decrease in the maximum specific growth rate values, when promoter strength was increased. The best performing strain displayed an 11% higher $Y_{ETH/S}$ value than the reference strain. We will measure ATPase activity in the engineered strains, in order to confirm that the phenotypes observed are due to increased ATPase activity. The strategy reported here presents the following advantages: 1) does not require evolutionary engineering after metabolic engineering; 2) can easily/quickly be implemented in different backgrounds; 3) can be finetuned by the use of promoters with different strengths; 4) does not depend on the nature of the sugar; 5) does not use heterologous genes. Acknowledgements: FAPESP, CAPES and CNPq (Brazil).

PS3-13: Spontaneous circadian rhythms in a cold adapted native *Aureobasidium pullulans*

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Rhythmic environments shaped the evolution of circadian timing systems capable of synchronizing endogenous biological processes to day/night cycles. This entrainment, critical for health and survival of individuals and populations, is based on transcriptional-translational negative feedback loops that generate close to 24hr oscillations in gene expression and physiology, even in the absence of external timing cues. Circadian systems are found from cyanobacteria to mammals, and though they involve different genes in diverse organisms, the basic mechanisms of cellular circadian timekeeping are similar in all known circadian clocks. The circadian system in fungi is well described, though mostly from the analysis of the filamentous fungus *Neurospora crassa* and in very specific growth conditions. It is not known if a molecular clock – as described for *N. crassa* – is present in yeasts, and if so, how circadian phenotypes in these organisms could be exploited biotechnologically. Working under the hypothesis that natural circadian traits are more likely to be retained by environmental strain isolates rather than their lab counterparts, *Aureobasidium pullulans* CRUB 1823, a psychrotolerant yeast with biotechnological potential, was isolated from natural sources, screened for spontaneous circadian phenotypes, and for expression of circadian clock genes (qPCR). When grown on solid media, colonies displayed daily formation of concentric rings with morphology and frequency dependent on light exposure. Rings were present in cultures grown in 12hr/12hr light/dark cycles and persisted in constant darkness, indicating that ring formation is controlled by an endogenous circadian clock and light. A gene expression analysis revealed that *A. pullulans* express homologs of the *N. crassa* clock-genes *frq*, *wc-1*, *wc-2*, and *vvd* suggesting a conserved circadian clock. Further work is being conducted to evaluate if the circadian clock in *A. pullulans* can modulate the expression of biotechnologically relevant genes and products including pullulan (used for production of edible biofilms), Aureobasidin A (fungicidal) and hydrolytic enzymes. Such knowledge could be used to develop efficient and economical strategies to improve production by using appropriate lighting schedules or timing of harvest. It will also broaden our knowledge about the general basis and evolution of fungal circadian clocks, in particular of cold adapted organisms, and their importance to fungal survival.

PS3-14: Feedback control of Snf1 protein and its phosphorylation is necessary for adaptation to environmental stress

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Snf1, a member of the AMP-activated protein kinase family, plays a critical role in metabolic energy control in yeast cells. Snf1 activity is activated by phosphorylation of Thr-210 on the activation loop of its catalytic subunit; following activation, Snf1 regulates stress-responsive transcription factors. Here, we report that the level of Snf1 protein is dramatically decreased in a *UBP8* and *UBP10* deleted yeast mutant (*ubp8*^Δ *ubp10*^Δ), and this is independent of transcriptional regulation and proteasome-mediated degradation. Surprisingly, most Snf1-mediated functions, including glucose limitation regulation, utilization of alternative carbon sources, stress responses, and aging, are unaffected in this strain. Snf1 phosphorylation in *ubp8*^Δ *ubp10*^Δ cells is hyper-activated upon stress, which may compensate for the loss of the Snf1 protein and protect cells against stress and aging. Furthermore, artificial elevation of Snf1 phosphorylation (accomplished through deletion of *REG1*, which encodes a protein that regulates Snf1 dephosphorylation) restored Snf1 protein levels and the regulation of Snf1 activity in *ubp8*^Δ *ubp10*^Δ cells. Our results reveal the existence of a feedback loop that controls Snf1 protein level and its phosphorylation, which is masked by Ubp8 and Ubp10 through an unknown mechanism. We propose that this dynamic modulation of Snf1 phosphorylation and its protein level may be important for adaptation to environmental stress.

PS3-15: Cellular localization of sun4p and its interaction with proteins of the yeast birth scar

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The predicted yeast glucanase, Sun4p is a member of the SUN family of proteins, homologous in their C-terminal domains. Sun4p has dual cellular localization to mitochondria and cell walls and is released from cells into the extracellular space (Kuznetsov et al., 2013). In the present study we show that Sun4p is a daughter-specific protein with cellular localization to birth scars - cell wall structures with unknown composition, which arise on cell surfaces after mother-daughter cell separation. The presence of Sun4p within birth scars and extracellular matrix indirectly depends on the Ace2p transcription factor. We determined the precise localization and co-localization of Dse2p, Dse4p and Sun4p within the yeast cell wall. We showed that the specific localization of Sun4p depends on the presence of Dse2p and that the localization of both Sun4p and Dse2p to birth scars depends on GPI-anchored Egt2p. Deletion or combined double deletion of any of these predicted glucanases leads to cell separation defects. We hypothesize that these proteins are parts of the septum destruction complex which localizes to the daughter side of the bud neck and the birth scar and are required for mother-daughter cell separation at late mitosis. Using a novel immunofluorescence approach to yeast birth scar visualization we found that Aim44p is necessary for correct new bud selection. A strain with a disrupted AIM44 gene showed a so called “budding-within-birth scar” phenotype where new buds always appear within birth scars, the zone restricted for budding. A similar phenotype is seen in a strain deleted for gene SWI5, encoding a transcriptional regulator of AIM44. The work was supported by GAUK 903313, GACR 13-08605S and Biocev (CZ.1.05/1.1.00/02.0109).

PS3-16: Hexose transporters of *Yarrowia lipolytica*

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Yarrowia lipolytica is an oleaginous yeast which potential as a lipid production platform from sugar-containing biomass or industrial co-products (such as molasses) is of importance. Only scarce experimental data on hexose uptake are available for this species and particularly no fructose transporter has yet been identified [1]. In silico studies tempting to point out candidate genes for hexose transporters, homologous to the HXT family of *S. cerevisiae*, produced contrasted results [2,3]. In order to identify hexose transporters of *Y. lipolytica*, we carried out systematic screening starting from the identification of 24 members of the Sugar Porter family in two strains of *Y. lipolytica*. First, six main hexose transporter gene products were identified in a functional complementation test using the hxt null strain of *S. cerevisiae* [4], and named Yht1 to Yht6. Among them, four (Yht1 to Yht4) were able to promote fructose transport. Subsequently, we identified physiologically active transporters in *Y. lipolytica* by constructing mutants affected in single gene (yht1 to yht4) or combination of them. Single deletion of YHT1 resulted in lack of growth at low fructose concentration (0.05 and 0.1%), suggesting its involvement in high affinity transport of this sugar. The yht1 yht4 double mutant led to growth defect on fructose but also on glucose and mannose. This result is on line with expression data, revealing that YHT1 and YHT4 are transcribed during growth on both fructose and glucose, whereas transcripts for YHT2, YHT3 were not detected. In conclusion, *Y. lipolytica* exhibits redundancy for hexose transporters, although to a lesser extent than *S. cerevisiae*. Under laboratory conditions, it uses mainly two transporters (Yht1 and Yht4), which revealed the first fructose transporters identified in *Y. lipolytica*. This work was funded by the Agence Nationale de la Recherche (Investissements d'avenir program ANR-11-BTBR-0003). Z. Lazar received an Agreen Skills Fellowship from the European Union in the form of (Grant agreement 267196; Marie-Curie FP7 COFUND People Program). Publication supported by Wrocław Centre of Biotechnology, program The Leading National Research Centre (KNOW) for years 2014-2018.

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PS3-17: Metabolite damage and repair in yeast

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One of the revelations of the genomic revolution is that thousands of proteins encoded by sequenced genomes remain without identified function. Sequence database analyses show that, even in a well-characterized organism like *Saccharomyces cerevisiae*, up to 2000 proteins have still incomplete functional annotations and that around 500 yeast genes seem to encode enzymes to which no clear molecular and/or physiological role has been assigned yet. Recent findings suggest that a possibly significant fraction of these enzymes of unknown function are involved in a process called metabolite repair. Abnormal metabolites are constantly generated inside the cell by unwanted chemical reactions or by enzymatic side reactions; they are useless at best and toxic at worst. Metabolite repair enzymes clear the metabolite pool of these non-canonical metabolites. Their importance is well illustrated through implication in disease processes. L-2-hydroxyglutaric aciduria for example, a severe human neurometabolic disorder, is caused by a deficiency in a metabolite repair enzyme. The latter normally prevents accumulation of a toxic side product formed by L-malate dehydrogenase. A series of other metabolite repair enzymes have been identified in mammals, but also in plants, invertebrates and microbes. I will describe how we identified the gene encoding NADHX dehydratase in yeast and how this led to the discovery of an additional enzyme that contributes to the removal of potentially toxic NADH and NADPH derivatives. NADHX, a hydrated form of NADH, can be produced in a side reaction catalyzed by GAPDH, but NADHX and NADPHX can also be formed spontaneously from NADH and NAPDH under conditions of low pH or increased temperature. Those hydrated forms of NAD(P)H can inhibit important metabolic enzymes and may therefore be toxic for the cell. Using yeast mutants deficient in the NADHX dehydratase, we could demonstrate for the first time that NADHX is formed endogenously in yeast and that intracellular levels of this unstable metabolite can be

modulated through environmental and genetic perturbations. I will also show results on the biochemical characterization of yeast homologs of the human metabolite repair enzyme D-2-hydroxyglutarate dehydrogenase. Targeted metabolomics analyses of yeast deletion mutants of these homologs generated intriguing and highly interesting results, that have led to the discovery of a new type of metabolite repair function in yeast.

PS3-18: YMR210w of *Saccharomyces cerevisiae* catalyses both triacylglycerol lipase and ester hydrolase activites and KO display accumulation of lipid bodies

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Yeast lipases and ester hydrolases involved in lipid metabolism also play a major role in signalling, cell cycle and survival. *YMR210w*, though mentioned as a member of *Eht1* and *Eeb1* gene clad [1], it was found to be involved in the production of ethyl octanoate and ethyl deconoate only in the absence of *Eht1* and *Eeb1* genes. Here we report, *YMR210w* as triacylglycerol (TAG) lipase along with ester hydrolyzing capacity. Sequence analysis of *YMR210w* protein revealed the presence of conserved motifs *GXSXG* and *HXXXD*. Phylogenetic analysis showed homology to Human *ABHD1,2,3* and *Drosophila CG3488* genes. To evaluate the biochemical functionality, *YMR210w* was expressed in *S. cerevisiae* using pYES2/CT vector and His-tag purified recombinant protein confirmed TAG lipase activity. *In-vivo* TAG content variation among Wildtype (WT), *YMR210w* knock-out (Δ) and *YMR210w* over-expressed (OE) strains substantiate the *in-vitro* TAG lipase activity. Ester hydrolase activity was confirmed with pNP-acetate, pNP-butyrate and pNP-palmitate. GC-MS lipid-profiling of *YMR210w* (Δ) showed an increase in the 15:0 Pentadecanoic acid by 76% among the total lipids. Phospholipid, Erucic acid 22:1 (Δ^{13}) showed 43% add on while sterol esters showed significant changes with 16:0 hexadecanoic acid augmentation by 80% and 18:0 Octadecanoic acid by 165% when compared to WT. Increased accumulation of lipid bodies was also observed in *YMR210w* (Δ) strain when compared with WT cells by TEM analysis supporting the increase in the sterol ester and TAG content. Based on the above results, *YMR210w* is identified as novel cytosolic TAG lipase and ester hydrolase.

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PS3-19: Identification of critical residues for transport activity of Acr3p, the *Saccharomyces cerevisiae* As(III)/proton antiporter

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The proton-driven antiporter Acr3p from budding yeast mediates extrusion of the toxic metalloids arsenic and antimony out of yeast cells. We have previously found that Cys151 located in the middle of the fourth transmembrane segment (TM4) is critical for antiport activity, suggesting that As(III) might interact with a thiol group during the translocation process. In order to identify functionally important residues involved in As(III)/proton exchange, we first confirmed experimentally that the yeast Acr3p has ten transmembrane helices; then we performed a systematic alanine-replacement analysis of charged/polar and aromatic residues that are conserved in the Acr3 family and located in transmembrane segments. Nine residues (Asn117, Trp130, Arg150, Trp158, Asn176, Arg230, Tyr290, Phe345, Asn351) were found to be critical for proper folding and trafficking of Acr3p to the plasma membrane. In addition, we found that replacement of highly conserved Phe266 (TM7), Phe352 (TM9), Glu353 (TM9) and Glu380 (TM10) with Ala abolished transport activity of Acr3p, while mutation of Ser349 (TM9) to Ala significantly reduced the As(III)/proton exchange, suggesting an important role of these residues in the transport mechanism. Detailed mutational analysis of Glu353 and Glu380 revealed that the negatively charged residues located in the middle of transmembrane segments TM9 and TM10 are crucial for antiport activity. Based on the topological similarity between the yeast Acr3p and two bacterial homologs of the bile acid sodium symporter ASBT, belonging to the same bile/arsenite/riboflavin transporter (BART) superfamily, we propose that Acr3p exhibits a fold and transport mechanism similar to that commonly found in secondary active transporters.

PS3-20: Transcription regulators Gcn4p and Hac1p in yeast colony development**Jana Marsikova**¹, Michaela Schierova¹, Ladislava Hatakova¹, Libuse Vachova², Zdena Palkova¹¹Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Czech Republic; ²Institute of Microbiology, ASCR, v. v. i., Prague, Czech Republic

The unfolded protein response (UPR) and the general amino acid control (GAAC) are two well-established pathways in *Saccharomyces cerevisiae*. The UPR is activated in response to different physiological and environmental stresses, which cause an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER), a condition termed ER stress. The bZIP transcription factor Hac1p plays a central role in the yeast UPR and contributes to activation of the expression of approximately 400 genes that promote cell survival and adaptation. The GAAC network is induced by amino acid starvation and by other changes in nutrient availability. Gcn4p that regulates transcription of amino acid biosynthetic genes represents the key activator of the GAAC. Hac1p is required for induction of Gcn4-responsive promoter elements and supports Gcn4p activity during amino acid starvation. Moreover, Hac1p and Gcn4p mutually influence their mRNA expression levels and Gcn4p is involved in *HAC1* transcription in both haploid and diploid cells. Furthermore, Hac1p and Gcn4p act on identical promoter elements of the *FLO11* and are required in diploids to induce *FLO11* expression [1]. Flo11p is the cell surface glycoprotein (flocculin) involved in pseudohyphal growth and required in surface adhesion and proper formation of structured architecture of yeast colonies [2]. Here, we analyzed the effect of the *HAC1* and *GCN4* genes in development of different types of yeast colonies. Among others we show that the impact of *HAC1* deletion is significant even under non-stress conditions and causes a radical reduction of structured colony architecture. The *hac1Δ* strain exhibits a decreased vegetative growth rate, reduced cell attachment to the agar and an ineffective cell-cell adhesion resulting in decreased flocculation. In differentiated colonies Gcn4p regulation of expression of genes of amino acid metabolism occurs in specific cell subpopulations and also does not correlate with the level of starvation of specific cell types. The work was supported by GAUK 460214, GACR 13-08605S, SVV-2015-260209 and Biocev (CZ.1.05/1.1.00/02.0109).

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PS3-21: Two glycerol 3-phosphate dehydrogenases play an important role in glycerol production of *Candida versatilis* SN-18 under high glucose concentration**Daiki Mizushima**, Hisashi Iwata, Yuki Ishimaki, Jun Kato, Jun Ogihara, Takafumi Kasumi*Laboratory of Applied Microbiology and Biotechnology, Department of Chemistry and Lifescience, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan*

Candida versatilis SN-18 produces a significant amount of glycerol under high concentration glucose condition. Thus, *C. versatilis* SN-18 holds promise for the large scale fermentative production of glycerol. However, the mechanism and characteristics of glycerol synthesis by *C. versatilis* SN-18 remain poorly studied on the basis of molecular biology. We here investigated the metabolic correlations in *C. versatilis* SN-18 between the expression of genes involved in glycerol biosynthesis and the level of glycerol production under conditions of high glucose concentration. Since, glycerol 3-phosphate dehydrogenase (GPD) is considered a key enzyme of glycerol biosynthesis, two isogenes of *GPD* from *C. versatilis* SN-18 were cloned and sequenced. These intronless genes (*Cagpd1* and *Cagpd2*) were both predicted to encode a 378 amino acid polypeptide, and the deduced amino acid sequences mutually showed 76% identity. Interestingly, *Cagpd1* and *Cagpd2* were located tandemly in a locus of genomic DNA within a 262 bp interval. To our knowledge, this represents a novel instance of isogenic genes relating to glucose metabolism. In heterologous expression using a *gpd1Δgpd2Δ* double deletion mutant of *Saccharomyces cerevisiae*, *Cagpd1* and *Cagpd2* transformants complemented the function of GPD, with *Cagpd2* being much more effective than *Cagpd1* in promoting growth and glycerol synthesis. These results revealed that two isogenes of *Cagpds* satisfactorily coded protein of GPD. Under 20% (w/v) glucose condition, the cells of *C. versatilis* SN-18 were grown up until 3 days. From the gene expression experiment of both *Cagpds*, it was revealed that *Cagpd2* expression was constitutive, whereas *Cagpd1* expression was incrementally upregulated from up to 2 days. Extracellular glycerol was also rapidly elevated accompanied by upregulation of *Cagpd1* expression. Moreover, GPD activity was also increased from up to 3 days. These results revealed that at least both *Cagpds* were apparently involved in glycerol production in *C. versatilis* SN-18, in particular *Cagpd1*.

contributed to it much more.

PS3-22: Mrpl34p role in mitochondrial ribosome biogenesis in *Saccharomyces cerevisiae*

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Mitochondria are semiautonomous organelles which have their own genome. During evolution, the mitochondrial DNA (mtDNA) was severely reduced, but yet encodes some proteins - essential for the energy conversion into ATP- translated by specialized mitochondrial ribosomes (mitoribosomes). *Saccharomyces cerevisiae* mitoribosomes consist of a small subunit 37S - comprising 15S rRNA and 34 proteins - and a large subunit 54S - formed by 21S rRNA and at least 44 proteins. However, the knowledge of the mitoribosomes assembly is poorly characterized. Our goal was to discover how Mrpl34p, a component of the large subunit, contributes to mitoribosomal biogenesis. Since the null mutant [^] *mrpl34* shows a pleiotropic phenotype due to mtDNA loss, we sought to conduct the study by the use of yeast harboring thermosensitive alleles (ts). An important point to characterize a ribosomal protein is the identification of its intermediates during the formation of the ribosomal subunit to which the peptide belongs. We isolated the mitochondria from the both wild type and ts mutants grown at the restrictive temperature (37°C), and extracted the proteins in two conditions: in the presence of magnesium, that keeps the structure of 74S ribosomes, and in the presence of EDTA, which promotes the dissociation of 37S and 54S subunits. We added the samples onto a continuous sucrose gradient (0,3M to 1M), and after 3h of ultracentrifugation, 14 samples were collected, applied onto a polyacrilamide gel, eletrophorated and transferred to a nitrocellulose membrane which was developed by the use of antibodies against some ribosomal proteins. The results showed the ribosomal subunits scattered along the gradient fractions of the samples treated with EDTA, indicating the presence of assembly intermediates being accumulated by Mrpl34p-ts deficiency. The same procedure was performed with samples in the presence of magnesium. In this condition, using the wild type strain, markers for 54S subunit showed peaks between fractions 1 and 2. On the other hand, using Mrpl34p-ts mutant, the peaks were between fractions 3 and 4, further confirming the existence of a ribosomal assembly intermediates. Our results indicate that Mrpl34p is incorporated into ribosomal subunit 54S biogenesis at a later stage, which are in line with previous findings showing that bacterial L34 also has a role only at later stages of bacterial ribosome biogenesis.

PS3-23: Yeast tolerance to various stresses relies on the Trehalose-6P Synthase (Tps1) protein, not on trehalose

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Trehalose is a disaccharide commonly found in nature from bacteria to fungi and plants. Decades of observations have tied a close thread between stress protection and trehalose accumulation in numerous organisms. In the model yeast *Saccharomyces cerevisiae*, trehalose synthesis is performed by the *TPS* (Trehalose Phosphate Synthase) system. Deletion of *TPS1* gene, which encodes the first enzyme for trehalose biosynthesis, resulted in stress hypersensitivity [1,2]. However, *tps1Δ* cells exhibited apparently unrelated pleiotropic phenotypes. Since all previous studies relied either on correlation between trehalose accumulation and stress resistance, either on assessment of *tps1Δ* stress sensitivity, our goal was to reassess which one, from trehalose and/or the Tps1p *per se* may serve yeast cells to withstand stress exposure. We employed two complementary strategies, using a CEN.PK MAL+ strain, able to accumulated trehalose from exogenous supplies [3], and creating a catalytically inactive variant of Tps1 protein. We demonstrated that trehalose does not protect yeast cells from dying following exposure to high temperature, oxidative or desiccative stress. Our data clearly stated the role of Tps1p *per se* as a key element for survival against these stresses. Using robust RT-qPCR method we also showed that role of Tps1p in thermotolerance was not dependent upon Hsf1-dependent transcription activity. Finally, our results revealed that Tps1p was essential to maintain ATP levels during Heat shock [4]. We brought for the first time unbiased proofs that stress protection role of trehalose was overestimated, and that Tps1p *per se* is crucial in stress resistance. Altogether, our findings supported Tps1p as a new “Moonlighting protein”, with a key

regulatory function in energetic homeostasis essential for survival against adverse conditions, regardless of its enzymatic role.

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PS3-24: VDAC-SOD1 cross-talk: Effects on mitochondrial metabolism in porin-less yeast strain

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Cu/Zn Superoxide Dismutase (SOD1) represents the main antioxidant defense against ROS in eukaryotic cells and is located in cytosol and intermembrane space of mitochondria. It has been proposed that mutant SOD1, associated to the familial form of Amyotrophic Lateral Sclerosis, can inhibit VDAC1 in the outer mitochondrial membrane. VDAC is the most important pore-forming protein on the mitochondrial outer membrane that regulates the passage of metabolites between cytosol and mitochondria. In *Saccharomyces cerevisiae*, the lack of the endogenous VDAC1 (Δ por1) leads to an impaired growth on non-fermentable carbon sources at the restrictive temperature of 37°C. This feature makes Δ por1 cells a valid model for mitochondrial metabolism studies. Our data shows that transformation of Δ por1 yeast with human SOD1 completely restores the cell growth deficit on different non-fermentable substrates and re-establish the physiological levels of ROS. The increase of polarized mitochondria in the cells transformed with hSOD1 further confirms the reactivation of mitochondrial metabolism. Expression of the dismutase-inactive G85R hSOD1 provokes the same effects, suggesting that they are not related to the dismutase activity of the protein. Quantitative Real-Time PCR shows that several genes of the outer mitochondrial membrane are induced by hSOD1. It has been reported that, under stress conditions, SOD1 can act as a transcription factor. Therefore, we propose that the lack of VDAC1 is the stress factor that triggers the translocation of SOD1 to the nucleus and its action as a gene inducer. This is the first evidence of a direct coordination between VDAC and SOD1 and the first result explaining the VDAC isoforms redundancy in eukaryotic cell.

PS3-25: Study of fermentation capacity and alcohol production of a microbial consortium in different controlled environmental conditions

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The microbial consortium was composed of two *Candida* yeasts, which were inoculated in sequence; first the *Candida tropicalis* strain was inoculated in a medium based on nitrogen, mineral salts and a mixture of carbohydrates (51% glucose, 30% fructose, 8% galactose, 7% arabinose, 2% xylose and 2% sucrose). After 24 h of incubation at 35 °C, the *Candida glabrata* strain was inoculated and the experiment prolonged for at least 3 days at 35 °C, implementing agitation at 200 rpm [1]. Fermentations were performed at 3-liter-fermenter scale. The pH adjustment during the entire fermentation was tested. A consumption of 80.6% of total sugars and an alcohol productivity of 7.7 g / l.h was observed when pH was not corrected. In contrast, a consumption of 89.5%, highlighting the consumption of 80% of the content of pentoses and a productivity of alcohol of 7.6 g / l.h was observed when a continuous adjustment of pH to 4.5 was corrected. This treatment enhanced carbon sources assimilation. To improve productivity of alcohol an experimental design (2²) was performed with four treatments were agitation (200 and 300 rpm) and aeration (0.25 and 0.50 vvm) applied after *Candida glabrata* inoculation were evaluated. Both factors had a significant effect on alcohol production and in maximum productivity. The best results were obtained at 200 rpm, and 0.25 vvm, wherein the glucose, galactose, xylose and sucrose were consumed at 100% and the fructose and arabinose at 67 and 66% respectively and with 34.3 g of produced alcohol and a productivity of 15.3 g / l.h.

[1] Estrada Martínez R., Pacheco López N., et al. (2013) *Artículo I en Tecnologías para la Bioingeniería en*

México. ISBN: 978-607-609-059-6. (Edición electrónica) Pps: 10-14.

PS3-26: Development of a yeast cell factory for production of aromatic compounds: test case flavonoids production

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There is much interest in aromatic compounds in the chemical industry as these can be used for production of dyes, anti-oxidants, nutraceuticals and food ingredients, between the aromatic derived compounds flavonoids play a key role in the development of new drugs. Yeast is a widely used cell factory and it is particularly well suited for production of aromatic chemicals via complex biosynthetic routes involving P450 enzymes. In *Saccharomyces cerevisiae* the fluxes towards aromatic amino acids (L-tryptophan, L-tyrosine and L-phenylalanine) are strictly controlled on transcriptional and kinetic levels and therefore are difficult to manipulate. We engineered *S. cerevisiae* for increased production of aromatic compounds by eliminating degradation, up-regulating the key enzyme encoding genes and removing feed-back inhibition in the pathway. In order to test the strain performance we overexpressed heterologous pathways for production of different flavonoid compounds such as Coumaric acid and naringenin. We were able to produce flavonoids using glucose as carbon source, for some of the compounds we were able to obtain more than 5-fold higher concentrations in the engineered strain compared with the reference strain. In summary, we developed a *S. cerevisiae* platform strain suitable for production of aromatic amino acids-derived compounds.

PS3-27: Role of the sphingomyelinase Isc1p in the regulation of iron homeostasis

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Iron is an essential element for survival of almost all organisms. It is a component of several metalloproteins, containing Fe/S clusters and heme centers, which are involved in many cellular processes. Iron acquisition systems have to be highly regulated to assure a continuous supply of iron but simultaneously prevent its toxicity associated with the formation of hydroxyl radicals by the Fenton reaction [1]. The *Saccharomyces cerevisiae* inositolphosphosphingolipid phospholipase C (Isc1p) hydrolyses complex sphingolipids to produce ceramide, a bioactive sphingolipid. *ISCI* deletion is characterized by premature aging, oxidative stress sensitivity and mitochondrial dysfunction [2]. It also exhibits an up regulation of genes involved in iron uptake leading to increased levels of iron [2]. In this study, we investigated the role of Isc1p in the regulation of iron homeostasis. We observed, using a colorimetric assay [3], that *isc1Δ* cells already accumulate iron in a concentration three times higher at exponential phase compared to the wild type. The accumulated iron occurred in both the ferric and ferrous forms. Using a fluorescent probe for ferric iron, we found that in addition to the increased levels, *isc1Δ* cells also exhibited an altered distribution of iron within the cell. Unlike wild type, *isc1Δ* cells do not accumulate ferric iron in the vacuole. Instead, iron seems to be distributed throughout the cell. Additionally, iron accumulation in *isc1Δ* cells was abolished upon deletion of *AFT1* or expression of an inactive form of Aft1p [4], suggesting iron accumulation in this mutant is dependent on this iron-responsive transcriptional activator. Studies are undergoing to further characterize the regulation of iron homeostasis by Isc1p. This work was funded by FEDER (Fundo Europeu de Desenvolvimento Regional) through the program “Programa Operacional Fatores de Competitividade – COMPETE and by FCT (Fundação para a Ciência e a Tecnologia) through the project FCOMP-01-0124-FEDER-028210 (PTDC/BBB-BQB/1850/2012). VT was supported by an FCT fellowship

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PS3-28: Role of transcription factor KIMga2 in the regulation of fatty acids biosynthesis and cellular fitness in *Kluyveromyces lactis*

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Glucose repression is not the predominant regulation of carbon metabolism in *K. lactis*: regulation of glycolytic (*RAG1*) and fermentative (*KIPDC1*) genes is significantly dependent on oxygen availability and also lipid biosynthesis is regulated by hypoxia. We have focused our studies on the role of the hypoxic regulatory gene *KIMGA2*. We have found that the deletion of *KIMGA2* causes the loss of the hypoxic induction of some genes, especially those involved in lipid biosynthesis, and the reduction of transcription level of other metabolic genes. Transcriptional response of lipid biosynthetic genes to low temperature was affected in the mutant strain. The *klmga2Δ* strain also showed reduced growth rate and *rag⁻* phenotype, the latter is typically associated to glycolytic/fermentative defects. This phenotype was suppressed by unsaturated fatty acids (UFAs). The mutant strain also showed defects in mitochondrial morphology, respiration and catalase expression. Hypoxic shift in *K. lactis* generates induction of transcription of many genes. We showed that the hypoxic regulator KIMga2 has a role in this response. However, KIMga2 is also involved in mitochondrial/respiratory functions and oxidative stress suggesting a general role of this protein in regulating cellular fitness. The suppression by UFAs of the defects of the mutant strain indicate the importance of membrane functions in the mechanisms controlled by KIMga2. This work was partially funded by Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese. [Micolonghi et al. *Eukaryot Cell* 10:146-148 (2011). Micolonghi et al. *Microbiology* 158:1734-1744 (2012). Ottaviano et al. *FEMS Yeast Res* accepted (2015)].

PS3-29: The Lst4-Lst7 complex is a GAP for the Rag GTPase Gtr2 and is regulated by amino acid availability.

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The target of rapamycin complex 1 (TORC1) is a conserved protein kinase and master regulator of cell growth that responds to various intra- and extracellular stimuli including hormones and/or nutrients such as amino acids. Mutations in the TOR pathway have been implicated in several human diseases like for instance cancer or diabetes. The structurally conserved *Saccharomyces cerevisiae* vacuolar membrane-associated EGO protein complex (EGOC), which corresponds to Rag Ragulator in mammals and consists of Ego1, Ego3, Gtr1, and Gtr2 in yeast, mediates amino acid signals to TORC1. Within EGOC, the Rag GTPases Gtr1 and Gtr2 assemble into heterodimeric complexes and when Gtr1 and Gtr2 are respectively in their GTP- and GDP-loaded, signaling competent forms, they can promote TORC1 activation. The GTP/GDP loading status of GTPase proteins is often dependent on the activity of GTP exchange factors (GEFs) and GTPase-activating proteins (GAPs). While for Gtr1 a GEF (Vam6) and a GAP (SEACIT) have been identified previously [1-3], for Gtr2 such factors are still elusive. Recently, it has been demonstrated that the mammalian FNIP-Folliculin GAP complex promoted the GDP-loaded status of RagC/D, which are the closest homologs of Gtr2 in higher eukaryotes[4-5]. Here we will present our characterization in yeast of a similar protein complex composed of Lst4 and Lst7 that acts as a GAP for the Rag GTPase Gtr2. Moreover, we will also provide evidence that the Lst4-Lst7 GAP complex is regulated by amino acids and hence mediates amino acid signaling to control TORC1 via Rag GTPases.

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PS3-30: Role of Stl1 glycerol transporters in yeast osmotolerance

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Osmotolerance is the ability to grow in an environment with a high osmotic pressure. Yeast cells accumulate glycerol as a compatible osmolyte to survive the osmotic stress. Glycerol is produced to assure the redox balance in yeast cells. Its production is significantly increased under osmotic stress but synthesized glycerol is a small and uncharged molecule which diffuses across the cell membranes following its concentration gradient. Consequently, more glycerol is produced; more glycerol is lost via diffusion and the accumulation of the necessary amount counterbalancing the external osmotic pressure is very costly for the yeast cells. To accommodate quickly the optimum intracellular concentration of glycerol, *S. cerevisiae* cells employ two types of glycerol transporters. Fps1 channel serves to release surplus glycerol upon hypoosmotic shock, and Stl1 actively imports glycerol together with protons. Unfortunately, the *S. cerevisiae* Stl1 is repressed and inactivated by glucose thus it cannot be efficiently used for the reuptake of lost glycerol produced upon hyperosmotic stress when glucose is present. Osmotolerant yeasts are supposed to have very efficient glycerol uptake systems that diminish the necessity to produce large quantities of glycerol, as the glycerol lost by diffusion is taken up by these high-affinity glycerol-proton symporters. In this work, we studied the properties of putative glycerol transporters (homologs of *S. cerevisiae* Stl1) mediating the active uptake of glycerol in various non-conventional yeast species including osmotolerant *Zygosaccharomyces rouxii*, non-*cerevisiae* *Saccharomyces* species or a spoilage yeast *Dekkera bruxellensis*. Mutants lacking the *STL1* genes were prepared and their phenotype characterized in detail; and *STL1* genes were expressed in an osmosensitive *S. cerevisiae* strain. Our results show that all studied yeast species have active glycerol uptake systems regulated in different ways. When expressed in *S. cerevisiae*, the heterologous *STL1* genes render the cells more osmotolerant when a low amount of glycerol is added. Among the studied species, only *Z. rouxii* has two independent and regulable systems that contribute significantly to the osmotolerant nature of this yeast. Supported by GA CR 15-03708S and PITN-GA-2010-264717 Cornucopia.

PS3-31: Identification of the partners of Gup1, the yeast putative Hedgehog-like morphogen

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Microbes can form organized multicellular structures in which cells behave differently according to their shape and localization within the community. In the case of yeasts, the associated morphogenesis and differentiation has multiple meanings corresponding to colony or biofilm formation, to differentiation in pseudo/true hyphae, and to cell shape maintenance during budding, mating and sporulation in response to environmental cues. The *Saccharomyces cerevisiae* membrane-bound *O*-acyltransferase Gup1 is the yeast orthologue of mammalian *HHATL*, the negative regulator of Hedgehog morphogen secretion. The deletion of *S. cerevisiae* *GUP1* is associated with, namely, plasma membrane and cell wall structure, lipid metabolism, trafficking, cytoskeleton organization and budding pattern, and in *Candida albicans* with morphological switching, biofilm formation, virulence and antifungal resistance. The present work aimed at identifying and characterizing in *S. cerevisiae* the intracellular partners of Gup1, as a first step to unveil the molecular role of this protein and devise whether yeasts harbour any Hedgehog-like morphogenic pathway. Several proteins were described to putatively interact with Gup1. These have diverse cellular localizations, which point to the possible existence of different partners for Gup1 according to its multiple intracellular localizations previously found. Using co-immunoprecipitation assay, we report a novel physical interaction with Gup1 – the mitochondrial Porin (Por1). Por1 is a voltage-dependent anion channel required for maintenance of mitochondrial osmotic stability and mitochondrial outer membrane permeability. Accordingly, cellular fractionation and western blotting confirmed the mitochondrial localization of Gup1. We also observed that the absence of Gup1p seems to affect the cellular levels of Por1p.

Making use of single and double deletions of GUP1 and POR1, detailed phenotyping will unveil the common associated processes.

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PS3-32: Glutamate rewires yeast metabolism and positively affects biomass yield

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The yeast *Saccharomyces cerevisiae* is sensible to the nature and amount of available nitrogen-containing compounds. Different nitrogen sources are usually divided into preferred sources and "poor" ones. The preference in nitrogen source is manifested either quantitatively by an enhanced growth rate in media containing the preferred source or qualitatively by the ability of the preferred source to induce repression of genes required for catabolism of other nitrogen sources. Our research focused on studying the physiology of yeast growing on glutamic acid. In contrast with the high growth rate sustained, glutamic acid displays a mild repression. We compared the behaviour of cells growing on glutamic acid with cell exposed to a reference nitrogen source (ammonium sulphate). Along with physiological comparison, we acquired transcriptomic and metabolomic data for the two nitrogen sources. Cells growing on glutamic acid show a sharp increase in cell size during exponential growth the biomass yield and a large increase in biomass accumulation in stationary phase. In glutamate-grown cells, a large transcriptional remodelling and a significant alteration in exo- and endo-metabolome is observed in glutamate-grown vs ammonium sulphate-grown cells. Excess alpha-ketoglutarate is secreted, and a significant accumulation of C atoms from glutamate is detected into intracellular trehalose, suggesting a redistribution between glucose and glutamate for the formation of anabolic precursors. About 50% of glutamate-derived alpha-ketoglutarate is secreted in the medium in exponential phase. Transcriptional data and enzymatic activities support the hypothesis of enhanced gluconeogenesis based on reserve macromolecules. Following glucose exhaustion, net accumulation of lipids and consequent activation of peroxisomal genes is observed. Cells grown on glutamate show enhanced resistance to oxidative stress. The above described results together with ongoing investigation of the redox state of the cell, high resolution profiling of endo-metabolome and FBA modelling will allow system-level understanding of glutamate utilization as a nitrogen and carbon source.

PS3-33: The N-terminal acetyltransferase NatC has a role in cellular stress response in yeast

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Protein N-termini can be modified in various ways either during or after protein synthesis, and will consequently increase the proteome complexity. N-terminal acetylation is a widespread eukaryotic modification and denotes the transfer of an acetyl moiety from acetyl-CoA to the α -amino group to the very first amino acid of a protein. The irreversible event is catalyzed by an evolutionary conserved enzyme family of N-terminal acetyltransferases (NATs) with defined substrate specificities. NatC acetylates the iMet when it is succeeded by the hydrophobic residues; Ile, Leu, Phe or Trp. The enzyme complex consists of three subunits namely Naa30, Naa35 and Naa38. The acetylation reaction is carried out by Naa30 but all subunits are indicated to be required for NatC-activity. NatC had previously been associated with yeast ability to utilize glycerol as the main carbon source. In addition, the expression of Naa35 has been shown to be glucose-repressed. A recent study in *C. elegans* revealed that NatC is involved in modulating stress tolerance through the insulin/IGF1-1 signaling pathway. To address cellular and biochemical consequences of N-terminal acetylation mediated by NatC, we have studied the complex under various stress conditions. Here, we confirm the finding that NatC deletion strains have diminished growth in non-fermentable media. Furthermore the protein level of Naa35 is upregulated in the same conditions. Our studies also show that deletion of *NAA30* and *NAA35* results in decreased sensitivity to specific metal stress. Altogether these findings suggest that N-terminal acetyltransferase NatC may have a role

in cellular stress response, such as respiration.

PS3-34: Adaptation to aneuploidy in budding yeast: Struggling for balance

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Being a hallmark of cancer cells, aneuploidy is a problem with a lot of medical relevance. Most of human solid cancer resulted from erroneous mitosis and harbour aneuploidy. Aneuploidy imposes a severe disadvantage for cell growth and is very detrimental, it is also associated with many developmental defects. Nevertheless, cancer cells are able to adapt to the conditions of aneuploidy, therefore it represents a liability as well as a source of selective advantage for cancer cells. In present study we show that aneuploid budding yeast are able to adapt to aneuploidy by multiple ways. A key feature of the aneuploid condition is its impact on protein homeostasis. To get insight into the consequences of aneuploidy on the proteostasis, we look at the effects of gaining the additional chromosomes in haploid yeast cells. Proteins are produced from the genes contained within chromosomes, and so cells with too many chromosomes produce too many of some proteins. How do these cells cope with this excess? The precise synthesis and turnover of proteins is vital. Intracellular quality control systems monitor proteins, and then misfolded proteins are cleared through specialized degradation pathways. Here we demonstrate how protein quality control machinery is deployed in meiotically generated multi-chromosome aneuploids. We checked quality control substrate processing in these strains and analyzed the kinetics of degradation for several cytosolic quality control substrates and ER-associated protein degradation (ERAD) substrates. The results suggest that aneuploids cope with the stress and continue to degrade the misfolded substrate more efficiently than wild type. We next verified the capacities of aneuploid cells to fold proteins. Interestingly, aneuploids display more than two times increased capabilities to fold proteins although chaperone's bunch is not altered. To find out how the aneuploids equip themselves to handle the enhanced protein load we performed ribosomal footprinting and deep sequencing of ribosome-protected mRNA fragments of aneuploid strains and wild-type cells. Taken together, we have a strong evidence for the translational control mechanisms taking over the attenuation of protein levels, and specific cellular feedback to the presence of additional chromosome could be a contributing factor.

PS3-35: Regulatory mechanism and physiological role of the flavoprotein Tah18-dependent Nitric Oxide synthesis in yeast

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Nitric oxide (NO) is a ubiquitous signaling molecule involved in the regulation of a variety of cellular functions in mammalian cells (protection against pathogens, blood pressure regulation, and nerve cell transmission). In the yeast *Saccharomyces cerevisiae*, the synthetic mechanism and the physiological role of NO remain unclear due to the lack of mammalian NO synthase (NOS) orthologues in the genome. Previously, our laboratory reported that NO is synthesized dependent on the flavoprotein Tah18 from L-arginine via NOS-like activity under high-temperature stress conditions. Here, we show the regulatory mechanism and physiological role of Tah18-dependent NO synthesis in yeast. Tah18 is an essential flavoprotein required for the cytosolic Fe-S protein biogenesis by interacting with the Fe-S protein Dre2. Recently, we found that the cellular NOS activity increases in proportion to a decrease in the Dre2 protein level. Furthermore, NO synthesis under oxidative stress condition (H₂O₂) was inhibited by treatment with a mammalian NOS inhibitor, NG-nitro-L-arginine methyl ester (NAME), or by conditional knockdown (KD) of the TAH18 gene, indicating that yeast NOS activity is dependent on Tah18. Interestingly, our biochemical analysis exhibited that oxidative stress conditions induce the Tah18-Dre2 complex dissociation, leading to the generation of NO. These results suggest that Dre2 is a negative regulator of Tah18-dependent NOS activity by interacting with Tah18. Although TAH18 KD cells showed higher cell viability than wild-type cells (WT), NAME treatment recovered cell viability of WT. Interestingly, TAH18 KD cells showed less NO production than WT in response to oxidative stress, suggesting that excess NO produced by Tah18-dependent NOS activity induced cell death, in accordance to the previous report. On the other hand, cell growth under non-stress condition was delayed by disruption of the YHB1 gene encoding NO detoxification enzyme only in DRE2 KD, but not in WT and TAH18 KD cells. From these results, we propose the regulatory mechanism of Tah18-dependent NO synthesis in response to oxidative stress. The Tah18-Dre2

complex normally participates in the cytosolic Fe-S protein biogenesis and Dre2 inhibits Tah18-dependent NOS activity to maintain intracellular NO level within its non-toxic level. Changes in redox state of Dre2, which is suggested to be a sensor protein for oxidation level, may be important for releasing Tah18 required for NO synthesis.

Poster Session 4: Mutagenesis, DNA damage and repair

PS4-1: Characterization of Rdh54 functions in mitosis

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The *Saccharomyces cerevisiae* Rdh54 protein is a chromatin remodeler that is required for efficient recombinational DNA repair and other aspects of genome integrity maintenance, including checkpoint adaptation and chromatin regulation. Despite being classified as a recombination protein, Rdh54 shows constitutive localization to the kinetochore, a macromolecular protein assembly that mediates the attachment of chromosomes to spindle microtubules. Recently, we have also shown that Rdh54 localizes to structures bridging sister chromatids during chromosome segregation in anaphase. These features suggest a role for Rdh54 in mitosis, but its exact involvement remains to be uncovered. In this study, we map the functional protein-protein interaction network of Rdh54 using a bimolecular fluorescence complementation (BiFC) assay. Using this technique we have identified a number of previously unknown interaction partners of Rdh54 pointing to its role at kinetochores and the mitotic spindle. We show that loss of *RDH54* results in increased sensitivity to the microtubule-depolymerizing drug benomyl, indicating that Rdh54 might be required for the spindle assembly checkpoint (SAC). Deletion of the spindle length regulating *KIP3* gene leads to an increased number of Rdh54 bridges and its localization to the spindle midzone. Rdh54 bridges are also induced by replication stress, suggesting that Rdh54 might be involved in both the regulation of spindle dynamics and processing of DNA anaphase bridges. Further, we find that Rdh54 and the Rad51 recombinase interact at the centromeric region as well as the mitotic spindle and that the *rad51* gene deletion causes an increased localization of Rad52 foci to the centromeric region. Therefore, we speculate that Rad51 might be important for the maintenance of centromere integrity.

PS4-2: Yeast-based assays for CRISPR/Cas9-mediated homologous recombination

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A CRISPR/Cas9 model assay was developed to study the efficiency of inter-chromosomal recombination mediated by short sequence homology. The approach is based on a double-reporter split assay in haploid yeast. The *ADE2* (chr. XV) and *TRP1* (chr. IV) loci were engineered to contain split constructs where both coding sequences were broken in two portions generating a 100nt duplication at each break site. The split reporters were constructed *in trans* (*i.e.* 5'ad-rp1 on chr. XV and 5'tr-de2 on chr. IV) to study inter-chromosomal recombination (herein referred to as ICR). The target site for a validated CRISPR/Cas9 sequence guide RNA (sgRNA) was then embedded in the split reporter within the *ADE2* locus (*i.e.* 5'ad-sgRNAtarget-rp1). A 100nt homology region corresponding to the sequence downstream the 3' portion of *ADE2* was also placed in the 3' portion of the split reporter on chr. IV. No homology is instead present among the 5' sides of the two split reporters. Thus, in this assay, Cas9 would induce a double strand break at the specific targetable sequence in the engineered chr. XV location and the formation of a functional *ADE2* gene, but not *TRP1*, can be expected through ICR mediated by the short homologies embedded in the two engineered loci. The sgRNA was constitutively transcribed while Cas9 was expressed using an inducible *GAL* promoter, from two available plasmids. Double transformants were selected on suitable glucose plates and the expression of Cas9 was induced by culturing cells for 6 hours on galactose media. Initial experiments indicated that the frequency of inter-chromosomal recombination leading to *ADE2* colonies was $\sim 6 \cdot 10^{-4}$ while no *TRP1* colonies were obtained (frequency $< 10^{-6}$), as expected. The formation of *ADE2* colonies was strictly dependent on the induction of Cas9 and the reconstitution of an intact *ADE2* gene was verified by colony PCR. The impact on the frequency of ICR events of deleting genes involved in NHEJ and HR (DNL4, ELG1, KU70, KU80, LIF1, NEJ1, SGS1 and XRS2) is being investigated, as well as the use of donor molecules to observe homologous recombination events leading to a reconstituted *TRP1* reporter. An equivalent approach has been developed to study single strand annealing events. These yeast-based assays can represent a versatile toolbox to optimize strategies for Cas9-mediated homologous recombination and gene conversion events that could potentially be translated to haploinsufficient genetic disorders in higher eukaryotes.

PS4-3: Induction of gene and structural mutations by heavy ions in yeast

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The modern study of the mutagenic action of accelerated heavy ions is of basic importance for planning long-term space flights - for example, to Mars. In space, organisms are exposed to ionizing radiation - mostly, heavy charged particles with high linear energy transfer (LET) and relative biological effectiveness (RBE). For this reason, research was started on the effect of heavy ions in a wide LET range on prokaryotes and eukaryotes. The use of a unicellular eukaryote — the yeast *Saccharomyces cerevisiae* — allows studying regularities in the induction of different types of mutations including gene mutations. A set of genetic systems for the detection of mutagenic events was used. Haploid yeast cells were irradiated by ^{60}Co γ -rays (1.17 MeV, 0.7 Gy/min) and accelerated ^{11}B ions (45 and 60 keV/ μm) at the doses up to 100 Gy. The cells were more sensitive to heavy ions than γ -rays. The relative biological effectiveness (RBE) coefficients of heavy ions estimated on the basis of their lethal action were 2.1 for ^{11}B (45 keV/ μm) and 2.3-3.5 for ^{11}B (60 keV/ μm). The gene mutation frequency increased linearly after irradiation by γ -rays and heavy ions. ^{11}B (60 keV/ μm) induced base pair substitution with the same RBE (1.0); frameshift mutation RBE increased (1.7); but total gene mutagenesis for the *CANI* gene was significantly inhibited (RBE 0.2). So the share of different mutations changed with increasing LET. Studying the dependence of chromosome rearrangement induction on the irradiation dose showed that in haploid yeast cells ^{11}B ions were more efficient than γ -rays in inducing intrachromosomal homologous recombination between inverted repeats (RBE - 20.9, ^{11}B - 60 keV/ μm) and extended deletions emerging in the plasmid vector due to DNA double-strand break repair by illegitimate end joining (RBE - 15.5 and 30.2, ^{11}B - 45 and 60 keV/ μm , respectively). The mutagenesis curve after γ - and heavy ions irradiation was linear-quadratic for recombination frequency and linear for deletions in the plasmid. An analysis of the loss of the largest yeast chromosomes IV (1554 kbp) and VII (1091 kbp) under γ -ray irradiation in disomic haploid strains showed the instability of these chromosomes. Chromosome IV was less stable than chromosome VII. The chromosome loss frequency curve was linear for γ -rays and linear-quadratic for ions ^{11}B (45 keV/ μm). Chromosome loss was induced very efficiently by γ -rays and yet more efficiently by ^{11}B ions.

PS4-4: The Rad27 nuclease is involved in preventing mitochondrial mutagenesis and is required for efficient mitochondrial homologous recombination in *S. cerevisiae*

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Replication and repair of mtDNA require the involvement of nuclelease activities to process different mtDNA intermediate structures and to maintain the stability of the mitochondrial genome. One of the nucleases that is implicated in mtDNA repair is Rad27. It was previously reported that deletion of the *RAD27* gene results in the mitochondrial mutator phenotype. However, the mechanism underlying the enhanced mitochondrial mutagenesis conferred by *RAD27* inactivation is not understood. To get more insight into the mitochondrial mutator phenotype caused by the lack of the Rad27 nuclease, mutations arising in *rad27Δ* deletion strains were analyzed. The frequency of mutations conferring resistance to erythromycin was used as a measure of mtDNA mutability. These mutations are acquired through specific changes in the mitochondrial 21S rRNA gene and acquisition of resistance to this drug is a standard method of measuring mtDNA point mutagenesis. We have found that while after standard 7 day-long incubation on the selective medium the frequency of erythromycin resistant (E^R) mutants increased only slightly in the *rad27Δ* mutant, as compared to wt strain, a prolonged incubation of the mutant cells on the erythromycin medium resulted in one order of magnitude enhancement in the occurrence of E^R . The sequence analysis of the mitochondrial E^R mutations showed that in contrast to mutations in wt strain, where mostly AT \rightarrow GC transitions and AT \rightarrow TA transversions were found, a remarkable GC \rightarrow AT shift was observed in late E^R mutants arising in the *rad27Δ* strains and about 75 % of them were due to GC \rightarrow AT transitions. The latter transitions are known to be frequently caused by either spontaneous or by oxidative deamination of cytosine. Studies on the genetic relationships between deficiency of Rad27 and other pathways known to be involved in the mtDNA repair revealed that a partial inactivation of the Msh1-dependent pathway,

conferred by the *msh1-R813W* allele, caused a synergistic increase in the frequency of E^R mutants, suggesting that Rad27 nuclease and Msh1 act on the same pre-mutagenic lesion in mtDNA, but operate in different repair pathways. Finally, we found that *rad27Δ* mutants exhibit a significant decrease in the efficiency of allelic mitochondrial homologous recombination (mtHR) estimated within *arg8^m* reporter gene. The deficiency in mtHR, which plays an important role in the yeast mtDNA repair, can at least partially explain the mitochondrial *rad27Δ* phenotype.

PS4-5: Understanding the function of mitochondrial tim9 and tim10 and their degradation by the i-AAA protease Yme1

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Tim9 and Tim10 are essential homologue proteins of the mitochondrial inter membrane space (IMS). They act as ATP-independent chaperones for precursors destined for the inner and outer membranes of mitochondria. Both proteins are synthesised in the cytoplasm, and are characterised by the presence of four conserved cysteine residues, arranged in two CX3C motifs. Thiol-disulphide redox-switch of the cysteine residues plays a key role during the biogenesis of these proteins. In the IMS, the oxidised proteins form a stable hexameric Tim9-Tim10 complex in a multistep assembly process driven by electrostatic and hydrophobic interactions. In terms of the functional mechanism, it is elusive how these proteins mediate their substrates across the aqueous environment of the IMS to their destination, and it is unclear how homeostasis of these proteins is maintained in the IMS. We examined the requirement and role for the individual disulphide bonds and key hydrophobic residues of Tim9 on cell viability, the complex formation and stability using yeast genetic, biochemical and biophysical methods. Loss of the Tim9 inner disulphide bond led to a temperature-sensitive phenotype and degradation of both Tim9 and Tim10. The growth phenotype could be suppressed by deletion of the mitochondrial i-AAA protease Yme1, and this correlates strongly with stabilisation of the Tim10 protein regardless of Tim9 levels. Similarly, loss of two key hydrophobic residues of Tim9 also led to a temperature-sensitive phenotype, but not degradation of Tim9 and Tim10 or the growth phenotype can be suppressed by deletion of Yme1. Furthermore, effects of the mutants on the complex assemble and stability of the proteins was characterised using purified proteins. Our results suggest that (i) formation of both disulphide bonds is not essential for Tim9 function, but it can facilitate the formation and increase the stability of the hexameric Tim9-Tim10 complex. (ii) The primary function of Tim9 is to protect Tim10 from degradation by Yme1 via assembly into a stable Tim9-Tim10 complex. (iii) Formation of the hexameric Tim9-Tim10 complex is important for stabilisation of the individual Tim9 and Tim10, rather than the function of these proteins.

PS4-6: Nrl1 is a new spliceosomal factor involved in maintenance of genome stability of *S. pombe*

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Genomic instability is defined as a process prone to genomic changes or an increased propensity for genomic alterations. Intron splicing is a widespread event with important, yet elusive, implications for the regulation of eukaryotic gene expression and genome stability. Here, we show that the evolutionarily conserved protein Nrl1 of the fission yeast *S. pombe* binds to spliceosome complexes. We propose that proper regulation of splicing through Nrl1 is essential to prevent the accumulation of genome-threatening RNA-DNA hybrids, referred as to R-loops, thus safeguarding genome integrity. Since Nrl1 is conserved in higher eukaryotes including humans, our study paves the way to a deeper understanding of the mechanism and functional significance of metazoan splicing.

PS4-7: A systematic screen to uncover synthetic spore lethality with *spp1Δ* identifies the ZMM pathway

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Meiosis is a specialized cell division required for sexually reproducing organisms to produce four haploid gametes from one diploid precursor cell. During meiosis, one round of chromosome replication is followed by two rounds of segregation, the first separating homologous centromeres, the second one sisters. In order to be able to segregate homologous chromosomes to different daughter nuclei during the first meiotic division, homologs need first to be identified, which occurs by sequence matching in the course of the repair of self inflicted DNA-double strand breaks (DSBs). Once identified, homologs pair and connect physically via chiasmata, formed by crossovers (COs) in the context of sister chromatid cohesion. DSBs occur in promoter regions of actively transcribed genes on loops protruding away from the axis, whereas most of the DSB machinery localizes to cohesin binding sites at the axis. Spp1 could act as a potential tether between loop and axis, because it binds to H3K4me3 via its PHD finger and at the same time to Mer2, a member of the DSB machinery. Indeed, mutants lacking Spp1 display decreased overall levels of DSBs with strong DSB hotspots dramatically reduced; despite the substantial overall DSB decrease, *spp1Δ* produces near 100% viable spores. In order to expose the compensatory mechanism responsible for high spore viability and the creation of some novel hotspots found in *spp1Δ*, we carried out a systematic synthetic spore lethality screen between *spp1Δ* and the deletion library of *S. cerevisiae*. To increase sensitivity and to reduce noise, spore viability was assayed by high throughput flow cytometry, using a newly developed GFP based haploid reporter system. This analysis revealed synthetic defects between *spp1Δ* and several members of the ZMM pathway. It seems, though, that it is not *spp1Δ*'s reduced number of DSBs that leads to synthetic defects with zmm-mutants. A model of how *spp1Δ* affects the ZMM pathway will be discussed.

PS4-8: The *Saccharomyces cerevisiae* Swi6 is a genome maintenance protein, required especially after DSB stress

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The Swi6 protein is a member of two transcriptional complexes: SBF and MBF, that are necessary for expression of the genes responsible for the transition of cell cycle checkpoint: START point (G1/S), and the entrance into mitosis (G2/M) [Ferrezuelo et al. 2011; Shohat-Tal & Eshel., 2011]. Through the red-ox state of its Cys404 Swi6 works as a cellular red-ox sensor [Chiu et al. 2010]. The Swi6 controls expression of the genes involved in various cellular stresses, such as cell wall stress, oxidative stress, UPR stress and ER stress [Shyang Fong et al. 2008; Levin, 2005; Scrimale et al., 2009]. The strains lacking the *SWI6* gene exposed to environmental stress display delayed entry into the S phase of the cell cycle. We found the *SWI6* gene in several global screens performed in our lab, in which we were looking for strains of *S. cerevisiae* that are mutators or that are oversensitive to double strand breaks resulting from zeocin treatment or endonucleases overexpression. Here we show that *SWI6* deficient strains:

- 1- are oversensitive to various genotoxic agents;
 - 2- display decreased ability to repair DNA damage provoked by zeocin;
 - 3- display changes in spontaneous mutagenesis level;
 - 4- show abnormalities in DNA content;
 - 5- demonstrate anomalies in nucleus morphology and/or in number of nuclei per cell;
 - 6- exhibit higher than wild type strain frequency of nucleoli abnormalities;
 - 7- show increased number of P-bodies;
 - 8- moreover, sensitivity to zeocin is partially rescued by vanillin.
- Our results suggest that Swi6 plays a role in genotoxic stress response and in genome maintenance processes via

control of rDNA expression and availability of the rRNA pool. Cells containing the Swi6-C404A altered variant, which lost red-ox state sensor ability, partially complement DNA stability related phenotypes of *swi6Δ* cells. Moreover, our data show that the phenotypes displayed by cells with disturbed Swi6 content or function are also dependent on the cell ploidy.

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PS4-9: Circular DNA as drivers for gene amplification

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Extrachromosomal circular DNA (eccDNA) in eukaryotic cells causes genetic variation through replication and missegregation. Recently, our laboratory has developed a new sensitive method for purification of circular DNA in yeast, the Circle-Seq method. By this method we have found thousands of different eccDNA's that carry one or more genes, including the [HXT6/7^{circle}]. As the hexose transporter genes HXT6 and HXT7 have previously been found to make amplifications under glucose limitations, we hypothesize that circular DNA could be intermediates of amplifications through their integration into the chromosome. To investigate this possibility, we used glucose limited chemostat cultures to select for HXT6/7 amplifications. The trajectory of [HXT6/7^{circle}] and chromosomal HXT6/7 amplifications in evolving yeast populations, was followed by inverse PCR and Circle-Seq methods. Additionally, tags were inserted between the two hexose transporter genes to serve as a marker for detection of circle reintegration into the chromosome. The tagged strain was mated to the *kar1-15Δ* strain, which is unable to make nuclear fusion and will only allow small molecules, such as the [HXT6/7^{circle}], to be transferred between the nuclei in the mating cells. Detection of the tag in recipient cells, *kar1-15Δ*, will be a result of [HXT6/7^{circle}] integration in the HXT6-HXT7 locus and will proof that eccDNA participates in gene duplications.

PS4-10: Sae2 function at dna double-strand breaks is bypassed by dampening Tel1 or Rad53 activity

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DNA double-strand breaks (DSBs) can arise either by exposure to environmental factors or by failures in DNA replication. DSB generation elicits a checkpoint response, whose key players are the protein kinases Tel1/ATM and Mec1/ATR, which propagate their checkpoint signals by phosphorylating the downstream checkpoint kinases Rad53 and Chk1 to couple cell cycle progression with repair. Yeast cells can repair DSBs using homologous recombination (HR), which requires the nucleolytic degradation (resection) of the 5' strands of the DSB ends. DSB resection is initiated by the MRX (Mre11-Rad50-Xrs2) complex, which acts together with Sae2 protein. Then, the nucleases Exo1 and Dna2 generate long stretches of ssDNA to allow strand invasion and pairing with undamaged homologous DNA templates. Mutations that impair Sae2 function result in resection defects and sensitivity to genotoxic agents. Furthermore, *sae2Δ* mutants show an unscheduled activation of Tel1 checkpoint kinase that, in turn, causes prolonged Rad53-mediated cell cycle arrest. These phenotypes suggest that Sae2 regulates both resection and checkpoint activation, but how these two events are connected to each other remain to be determined. We have identified Rad53-ss and Tel1-ss mutant variants that are able to suppress the DNA damage hypersensitivity and the resection defects of *sae2Δ* cells. We will present data regarding the molecular characterization of these variant and their suppression effects.

PS4-11: Evaluating the correspondence between genetic interactions and gene order of action in *S. cerevisiae*

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Although a molecular function has been revealed for over 80% of *Saccharomyces cerevisiae*, the specific position of genes within ordered biological pathways remains elusive. Most curated pathways are metabolic, and few regulatory pathways are considered completely known. Much of the information we have about connectivity and directionality of genes within pathways comes from genetic interaction analysis. Positive interactions, defined by double mutant phenotypes that are less extreme than expected, suggest that genes work in the same

pathway or in series. Masking and suppression relationships (also known as epistatic to relationships in Bateson's original sense of the word) are important subtypes of positive genetic interactions that can sometimes indicate gene order of action. Here we describe studies of the relationship between genetic interactions and functional gene order by extracting genetic interaction information of gene pairs with known order from large-scale Synthetic Genetic Array (SGA) data and small-scale DNA repair-related data. This represents the first systematic evaluation of the applicability of quantitative epistasis analysis to reveal gene order.

PS4-12: Improvement of the test-system for genotoxicology: Identification of a new class of genetical events detected in the alpha-test

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The alpha-test is a unique test-system providing the opportunity to determine the frequency of both inherited changes of different types and primary lesions in DNA *in vivo*. It is the only method which allows seeing the phenotypic effect of DNA damages before they will be repaired. The alpha-test is based on genetic system controlling the mating type in yeast *Saccharomyces cerevisiae*. The mating type of yeast strains is controlled by the *MAT* locus which is located on the right arm of chromosome III. The *MAT* locus determines the «a» or «α» mating types in heterothallic haploid yeast cells. Normally cells of opposite mating type ($\alpha \times a$) can mate. In the «illegitimate» mating ($\alpha \times \alpha$) the hybrids appear with low frequency. The hybrids appear due to mating type switching $\alpha \rightarrow a$ in one of the parent cells. The mating type switching could be caused by primary lesions, mutations and recombination events as well as loss of chromosome III or right arm of chromosome III. Different phenotypes of the hybrids in the alpha-test correspond to different molecular events of the mating type switching. It was suggested that some hybrids might appear as a result of genetical events outside of the *MAT* locus. In our work we investigated the sterile hybrids obtained in the «illegitimate» cytoduction (the modification of the alpha-test when one of the strains bears *kar1* mutation). It was predicted that such sterile cytductants contain mutations in the *MAT* locus. We demonstrated that the 2/3 of sterile sytductants do hold mutations in the *MAT* locus. The other part of sterile cytductants was heterogeneous in ploidy. One of the reasons for sterility is forming of di- and triploid cells a/a and $a/a/a$. Such cell may appear due to incomplete penetrance of mutation *kar1*. We have shown that polyploid cytductants arise with equal frequency among all detected classes and they do not distort the results of the alpha-test. To improve the accuracy of the alpha-test we recommend testing the hybrids for ploidy. Acknowledgements: Grant RFBR # 15-04-08625 and Research Grant of SPbU #1.38.426.2015.

PS4-13: Processing of the primary DNA lesions through the cell cycle and their phenotypic expression in yeast *Saccharomyces cerevisiae*

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In this study we have investigated the ratio between correct and error-prone processing of primary DNA lesions in relation with the cell cycle. We used an yeast-based assay that is called the alpha-test. The alpha-test is based on illegitimate mating α to α in heterothallic yeast *Saccharomyces cerevisiae*. The main advantage of the test-system is an ability to detect the own phenotypic expression of primary lesions before they are fixed by repair as well as results of the error-prone processing of primary lesions into gene mutations, recombination, gene conversion, chromosome and chromosome arm loss. Using the alpha-test we studied different mutagens, which cause specific DNA damage on background of different defects of repair. Our results indicate double-strand breaks, base modifications, cyclobutane-pyrimidine dimers together with 6-4 photoproducts may be detected in the alpha-test before DNA is correctly repaired. Mismatches may be seen in the alpha-test only after their transformation into genetic endpoints. Since yeast cells are able to mate only on the G1 stage, the own expression of those primary lesions which occur on the S, G2 or M stages and block DNA synthesis could be

missed in the alpha-test. To track primary lesions during the cell cycle we used a *rad52* mutant treated with camptothecin, a compound that induce double-strand breaks (DSBs) in the S stage of the cell cycle, and *apn1* mutant treated with methyl methanesulfonate, that accumulates DSBs in G1 and G2 stage. Our results show: the alpha-test allows to reveal phenotypic expression of primary lesions that occur mainly during G1 stage. Primary lesions arising on other stages of the cell cycle should be removed by repair before yeast cells get ready to mate in the next G1 stage. If the primary lesions are repaired inaccurately in the alpha-test we can only see resulting mutations. To investigate ability of primary lesions to pass cell cycle stages and express phenotypically we are going to use synchronized yeast cultures in the alpha-test. Acknowledgements: Grant RFBR # 15-04-08625 and Research Grant of SPbU #1.38.426.2015.

Poster Session 5: Telomeres, cell death and ageing**PS5-1: Investigating the role of Tbf1 in telomere homeostasis****Erin Bonnell, Raymund J. Wellinger***Université de Sherbrooke, Canada*

By differentiating chromosomal ends from internal breaks, telomeres prevent DNA damage checkpoint activation and provide protection from inappropriate DNA repair activity that could create genomic instability. The reverse transcriptase telomerase is responsible for telomere elongation and is constitutively active in *Saccharomyces cerevisiae*, making it an ideal organism to study telomere homeostasis. When an essential component of telomerase is removed, such as the templating RNA TLC1, cells enter replicative senescence after about 60 population doublings, with a small subset of the cellular population evading senescence via a recombination-dependent process. Previous studies have indicated that the time of onset of senescence can be influenced by many genetic factors, but not all mechanisms are known. *TBF1* is an essential gene that has been implicated in telomere homeostasis but its precise roles at telomeres still largely remains to be elucidated. It is known that Tbf1p binds T₂AG₃ repeats within subtelomeric regions, sequences in the majority of snoRNA gene promoters, as well as promoters of some protein-coding genes. While analyzing certain new *tbf1* mutant alleles, we discovered that the protein could have a much more direct role in telomere stability. Introducing a variety of *tbf1* mutants into strains that also lack telomerase (*tlc1Δ*), causes a dramatic change in the rate of senescence. We will discuss the ramifications of these and other results with the new *tbf1* alleles.

PS5-2: Developmental coordination of gamete differentiation with programmed cell death in sporulating yeast**Michael D. Eastwood, Marc D. Meneghini***Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada*

The gametogenesis program of the budding yeast *Saccharomyces cerevisiae*, also known as sporulation, employs an unusual internal meiotic division, following which all four meiotic products differentiate within the parental diploid cell. We showed previously that sporulation is typically accompanied by the destruction of discarded immature meiotic products through their exposure to proteases released from the mother cell vacuole, which undergoes an apparent programmed rupture. Here we demonstrate that vacuolar rupture contributes to a *de facto* programmed cell death (PCD) of the meiotic mother cell itself. Meiotic mother PCD is accompanied by an accumulation of depolarized mitochondria, organelle swelling, altered plasma membrane characteristics, and cytoplasmic clearance. To ensure that the gametes survive the destructive consequences of developing within a cell that is executing PCD, we hypothesized that PCD is restrained from occurring until spores have attained a threshold degree of differentiation. Consistent with this hypothesis, gene deletions that perturb all but the most terminal post-meiotic spore developmental stages are associated with delayed PCD execution. In these mutants, meiotic mother cells exhibit a delay in vacuolar rupture, and then appear to undergo an alternative form of PCD associated with catastrophic consequences for the under-developed spores. Our findings reveal yeast sporulation as a context of *bona fide* PCD that is developmentally coordinated with gamete differentiation.

PS5-3: Investigating Rad5 and the DDT pathway in telomere maintenance**Erin Henninger, Pascale Jolivet, Emilie Fallet, Mohcen Benmounah, Zhou Xu, Maria Teresa Teixeira**
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Telomeres protect chromosome ends by preventing their recognition as chromosomal breaks (DSBs) by the DNA damage response (DDR) pathways. However, to accomplish replication, telomeres are processed by components of the DDR machinery, resulting in progressive sequence loss at each cell division, a process counteracted by telomerase. In cells lacking telomerase, telomeres reach a critically short length, permanently activate the DNA damage checkpoint, and induce replicative senescence. Hence, telomeres provide a molecular alarm clock for the enumeration of generation numbers and the control of cell proliferation, which constitutes a major tumor suppression mechanism. Telomere progressive shortening results from the DNA end replication problem, oxidative DNA damage, and other DNA replication issues. The latter are due to potential secondary structures

formed at telomeres. While we have recently defined the contribution the DDR processing to telomere shortening, other issues ensuring the proper passage of the replication fork through telomeric repeats also become prominent in the absence of telomerase, shown by the activation of Mrc1 which signals replication stress. In this project, we showed that Rad5 associates with telomeres and is required for the viability of cells lacking telomerase in *Saccharomyces cerevisiae*. Rad5 is a ubiquitin ligase and DNA helicase that is proposed to promote replication fork regression or D-loop formation. It functions in conjunction with Ubc13 and Mms2 to polyubiquitylate monoubiquitylated PCNA (Pol30) in the DNA damage tolerance (DDT) pathway. This pathway allows the bypass of a damaged DNA template by the replication fork using a template switching mechanism or fork regression. We hypothesize that Rad5 is working to solve replication-associated problems that may continually arise at telomeres during replication. Therefore we set out to characterize Rad5 functions at telomeres. To this end, we are investigating the genetic interactions of *RAD5* in senescence onset, as well as the requirements for Rad5 recruitment to telomeres, and its effects on telomere structure and replication. Our results suggest that Pol30 ubiquitylation and/or sumoylation is involved in Rad5 activity at telomeres.

PS5-4: Mechanistic insight into yeast apoptosis in strains defective in cytoplasmic deadenylation and mRNA decay

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Strains of *Saccharomyces cerevisiae* lacking factors involved in 5' to 3' mRNA decay pathway (*DCP1*, *DCP2*, *DHH1*, *PAT1*, *LSM1* and *LSM4*) exhibit caspase dependent apoptosis and accelerated chronological aging. In the present study, yeast strains lacking mRNA decapping activation factors (*DCP2* and *LSM1*), cytoplasmic exosome function (*SKI2*) or cytoplasmic deadenylases (double deletion of *CCR4* and *PAN2*) showed typical markers of eukaryotic apoptosis such as increased cellular reactive oxygen species (ROS) levels, externalization of phosphatidyl serine, chromatin fragmentation and enhanced caspase activation in mid-log phase cultures. Among the yeast strains studied, *lsm1Δ* and *ccr4Δpan2Δ* mutants displayed strongest apoptotic phenotype followed by mutants lacking *DCP2* or *SKI2*. Slight apoptotic phenotype was observed in *ccr4Δ* mutants and cell death markers imperceptible in *pan2Δ* mutants. Double mutants of *CCR4/PAN2* were reported to be deficient in cytoplasmic deadenylation and accumulate all mRNAs tested thus far with longer poly(A) tails (>55 adenosines) suggesting longer poly(A) tail length of the accumulated mRNAs contribute to enhanced cell death in *ccr4Δpan2Δ* strain than *ccr4Δ* strain that accumulate mRNAs with 3' poly(A) tails of an intermediate length ~25-45 adenosines. Changes in transcript abundance of eIF4E and Pab1 in strains defective in deadenylation (*ccr4Δ* and *ccr4Δpan2Δ* mutants), decapping (*dcp2Δ* and *lsm1Δ* strains) or cytoplasmic exosome function (*ski2Δ*) suggest decapping antagonists contribute to stress responses accompanying enhanced cell death. In another study, analysis of contemporary c-terminal deletion mutants of yeast (*Saccharomyces cerevisiae*) *LSM1* suggest that even a loss of distal c-terminal domain of *LSM1* encoding 28 amino acid residues of Lsm1p could trigger 1.5 fold higher apoptosis than wild type control strain. Transcript levels as well as protein activity of mitochondrial citrate synthase 1 (*CIT1*), peroxisomal citrate synthase 2 (*CIT2*), aconitase 1 (*ACO1*) and isocitrate dehydrogenase 1 (*IDH1*) were considerably reduced in strains exhibiting apoptosis (*lsm1Δ*, *ccr4Δ* and *ccr4Δpan2Δ* mutants). The key enzymes of TCA cycle such as Cit1p, Cit2p, Aco1p and Idh1p form important components of retrograde signaling and yeast serves as valuable system to study interconnection on highly conserved cellular processes such as RTG signaling, target of rapamycin (*TOR*) signaling, lipotoxicity, mRNA metabolism and apoptosis.

PS5-5: Yeast acetic-acid induced programmed cell death resistance in raffinose is controlled by co-operation of *ADR1* and *CAT8* with the mitochondrial retrograde regulator *RTG2*

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Nutrient availability and the differentiation state determine cell fate in multicellular organisms. The yeast

Saccharomyces cerevisiae is a valuable model organism to study how nutrient availability determines cell fate in different growth conditions. In an attempt to understand how metabolism controls cell death and survival in actively dividing and proliferating cells, we have shown that exponential growing yeast cells in the presence of the preferred carbon source glucose (GLU-WT), which inhibits respiration through carbon catabolite repression (CR) pathway, undergo programmed cell death (PCD) in response to acetic acid (AA) treatment. On the contrary, yeast cells grown in raffinose (RAF-WT) are resistant to AA-PCD in a manner dependent on both the activation of mitochondrial retrograde (RTG) pathway, which senses mitochondrial dysfunction, and glucose de-repression of mitochondrial respiration. To study the relationships between CR and RTG pathway in yeast cell death and survival in response to AA, we compared WT cells and a set of yeast mutants lacking negative or positive regulators of either RTG or CR pathway as for their viability after AA treatment. We found that glucose-grown cells lacking *MIG1* and *HXK2*, which repress transcription of mitochondrial respiratory genes in the presence of glucose, undergo AA-PCD as GLU-WT cells, as judged by decrease in viability and increase in DNA fragmentation. On the other hand, differently from RAF-WT cells, raffinose-grown cells lacking transcription factors *ADRI* or *CAT8*, which are activated by alternative carbon sources, or *RTG2*, a positive regulator of RTG pathway, underwent AA-PCD. Cells lacking *HAP4*, the regulatory subunit of HAP complex active in raffinose, remain fully resistant to AA-PCD. Interestingly, double knock-out $\Delta adr1\Delta rtg2$ and $\Delta cat8\Delta rtg2$ cells were found nearly as much resistant to AA-PCD as RAF-WT cells. RTG pathway activation was studied in raffinose-grown WT and knock-out cells after AA treatment by analyzing the prototypical RTG-target gene *CIT2* mRNA level. Results showed that *ADRI* and *CAT8* are positive regulators of *RTG2*-dependent transcription and suggest that RTG and CR pathways co-operate in the control of cell fate through interaction between *RTG2* and *CAT8* or *ADRI*. *Acknowledgements.* Research was funded by grants from FIRB-MERIT BNE08HWLZ, the Italian Ministry of Economy and Finance to the CNR for the Project “FaReBio di Qualità” and project BioNet –PTP - PO Regione Puglia FESR 2000-2006.

PS5-6: Analyzing terminal phenotypes in *Saccharomyces cerevisiae* using Synthetic Genetic Array and High-Content Screening

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The study of cell death mainly focuses on delineating pathways involved in its execution. Just as important though, are the events upstream of these executioner pathways that play a role in the initiation of cell death. We are interested in expanding our picture of cell death from a focused view of execution to identifying linked and causal upstream events that lead to cell death. This chain of events that occur as a cell is dying is what we term a terminal phenotype, and will give us further mechanistic understanding of how cells die. To examine terminal phenotypes, we developed protocols that integrate the Synthetic Genetic Array (SGA) method with high-content screening (HCS) to enable the high-throughput, quantitative assessment of changes in subcellular morphology that occur as a cell is dying. We introduce fluorescent markers of key subcellular compartments into strains carrying temperature-sensitive (ts) alleles of essential genes, and then image live cells using high-throughput confocal microscopy. We introduced a panel of 23 diagnostic GFP markers, which monitor major compartments and functions in the cell, into a representative array of 384 different ts mutants using SGA, and imaged the resulting mutants over 24 hours. This analysis generates rich phenotypic profiles of essential gene mutants that capture the changes in subcellular morphology that span the time from first inactivation of the gene product to the death of the cell. Preliminary results indicate that terminal phenotypes are temporally dynamic, reflecting early changes in subcellular morphology that tend to be specific to the mutated gene and later defects that are more generally associated with cell death. Furthermore, mutants that display defects in multiple subcellular compartments point towards possible unexplored functional connections between subcellular compartments and structures. These assays will provide us with new information that will allow us to determine the mechanisms behind terminal phenotypes, and to gain further insight into the functional wiring diagram of the cell.

PS5-7: Subtelomeres influence telomere shortening-driven TERRA accumulation and replicative senescence in *Saccharomyces cerevisiae*

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In eukaryotes, telomeres determine cell proliferation potential by triggering replicative senescence upon telomere shortening in the absence of telomerase. In *Saccharomyces cerevisiae*, it is likely mediated by the first telomere that reaches a critically short length, which activates a DNA-damage-like response. How the signaling is modulated by the telomeric structure and context is largely unknown. Here we investigated how subtelomeric elements of the shortest telomere in the cell influence the onset of senescence. By comparing strains in which the pre-determined shortest telomere either harbors naturally occurring subtelomeric elements or lacks these elements altogether, we show that removal of subtelomeric regions accelerates the establishment of senescence. This effect is likely not due to differential Rad51-mediated homology directed repair activities at the different (sub)telomere variants. Furthermore, TERRA transcription is induced at both types of critically short telomeres, although levels are elevated in the absence of natural subtelomeric elements. Thus, subtelomeric elements become essential in the absence of telomerase, independently of being at the shortest telomere in the cell. Our results also demonstrate that telomeric transcripts from a telomere-proximal region greatly increase when the shortest telomere reaches a critical length, regardless of the presence of a native subtelomere or a dedicated TERRA promoter.

PS5-8: Tor1, Sch9 and PKA downregulation in quiescence rely on Mtl1 to preserve mitochondrial integrity and cell survival

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In this study we show that Mtl1, member of the Cell Wall Integrity pathway (CWI) of *Saccharomyces cerevisiae*, 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 (Target Of Rapamycin) and SCH9 deletion and less efficiently by Protein kinase A (PKA) inactivation. Mtl1 also plays a role in the regulation of both Bcy1 stability and phosphorylation, mainly in response to glucose depletion. In postdiauxic phase and in conditions of glucose depletion, Mtl1 negatively regulates TOR1 function leading to Sch9 inactivation and Bcy1 phosphorylation converging in PKA inhibition. Slt2/Mpk1 kinase partially contributes to Bcy1 phosphorylation, although additional targets are not excluded. Mtl1 links mitochondrial dysfunction with TOR and PKA pathways in quiescence, glucose being the main signalling molecule. This work was supported by the Spanish Ministerio de Economía y Competitividad through Grant BFU2012-31407.

PS5-9: Functional connection between the protein kinase Sch9 and the vacuolar proton pump in the yeast *Saccharomyces cerevisiae*

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The protein kinase Sch9 plays a central role in nutrient signaling and in regulating ageing in the budding yeast *Saccharomyces cerevisiae*. In this study a genome-wide synthetic genetic array (SGA) screening was performed to identify genes that genetically interact with *SCH9*. Gene Ontology (GO) analysis revealed the highly conserved vacuolar proton pump (V-ATPase) as one of the most significant hits. Using tetrad dissection and growth curve analysis, we established a general synthetic sick phenotype when combining deletion of *SCH9* with a dysfunctional V-ATPase. Furthermore, a significant phenotypic overlap between *sch9*^Δ and *vma*^Δ strains was

found. Besides this genetic interaction, we show that Sch9 also appears to physically interact with the V-ATPase. As Sch9 is an important determinant in regulating life span in yeast, we investigated what the cellular consequences of this genetic interaction were during chronological aging. Surprisingly, we discovered that Sch9 switches from pro-aging to pro-survival function when V-ATPase function is impaired. In conclusion, we provide evidence for a functional interaction between the protein kinase Sch9 and the V-ATPase. Given that both entities are well conserved in mammals it will be of interest to further elucidate these mechanisms in yeast and to see whether they are conserved in higher eukaryotes.

Poster Session 6: Yeast and industrial biotechnology: from fermented foods to cell factories**PS6.1: Study of the interactions of *Saccharomyces cerevisiae* with other *Saccharomyces* species in winemaking conditions**Javier Alonso-del-Real¹, María Lairón², Eladio Barrio², Amparo Querol¹¹Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Paterna, Valencia, Spain; ²Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de los Alimentos, CSIC, Paterna, Valencia, Spain; Departament de Genètica, Universitat de València, Spain

Wine consumers' new preferences, together with variations in grape musts due to climate change, bring new challenges to the wine industry. These challenges demand a global response in winemaking practices, including the development of new yeast starters, well adapted to these new requirements. In our group, research in that direction has been conducted focusing on the use of strains of non-conventional *Saccharomyces* species with interesting properties for the winemaking industry, such as a lower ethanol production combined with a higher glycerol synthesis, or the ability to ferment at low temperatures, which allows for a better aroma conservation of wines and avoids stuck and sluggish fermentations. Utilization of these organisms as starter cultures requires the study of their behaviours during their competition with the natural microbiota present in wine musts. Thus, our aim is to learn about interactions among yeasts resulting in the exclusion and domination of the inoculated yeasts during fermentation. Specifically, our approach consists on digging into the key points where these events take place, studying the global gene expression and metabolomics profiles of yeasts during competition compared to those when grown in pure cultures without competition. Until now, we have identified the main modifications in our yeasts growth behaviour and collected cells at these moments to carry out a global expression study by means of RNAseq. With the obtained data, we expect to find key genes or pathways involved in the *Saccharomyces-Saccharomyces* competition mechanisms.

PS6.2: Implementation of the Bridge Induced Translocation (BIT) technology for strain improvement of bioethanol-producing transgenic yeastBurcin Altun¹, Valentina Tosato¹, Carlo V. Bruschi^{1,2}¹Yeast Molecular Genetics laboratory, ICGEB, AREA Science Park, Trieste, Italy; ²CEI, Executive Secretariat, Trieste, Italy

Cellulose is one of the substrates that can be used for bio-ethanol production and *Saccharomyces cerevisiae* is the perfect organism for the fermentative conversion of glucose into ethanol. Since yeast does not break directly cellulose into its monomers, the genetic engineering of *S. cerevisiae* is an important step for the ethanol production process [1]. The present research deals with the implementation of the Bridge-Induced Translocation (BIT) technology [2,3] and yeast artificial chromosome (YAC) recombineering for yeast strain improvement. We applied BIT between a YAC that carries cellulose degradation genes and an endogenous yeast chromosome (IV). *Pichia stipitis* NBRC 10063 (CBS 6054) (for *BGL* and *EGC*) and *Trichoderma reesei* QM9414 (for *CBH*) strains were used as source for the cellulase genes. In order to optimize cellulases secretion successfully, we used a *PEP4* mutant yeast strain [4,5], which has decreased degradation of expressed recombinant proteins. Several tests were performed to check the breakdown of cellulose into glucose and its conversion into ethanol. The BIT technology offered us two advantages: the stabilization of the YAC, and an increased gene expression level consequent to the translocation event. In conclusion, the simultaneous utilization of YAC recombineering and BIT technologies allowed to optimize quickly the fitness of a yeast strain, a process that would otherwise take several time-consuming and labor-intensive rounds of genetic engineering. This work describes the successful improvement of a recombinant translocant yeast strain that is able to efficiently utilize raw cellulosic material as unique carbon source. We therefore demonstrated an efficient and economic conversion of cellulosic biomass into glucose in a GRAS microorganism, leading to interesting perspectives in bio-ethanol production.

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PS6-3: Metabolic engineering of *Saccharomyces cerevisiae* for enhanced production of acetoin**Sang-Jeong Bae, Sujin Kim, Ji-Sook Hahn***School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea*

Acetoin, also known as 3-hydroxybutanone, is widely used in food and cosmetic industry as taste and fragrance enhancer. Despite the presence of native pathway to produce acetoin in *Saccharomyces cerevisiae*, its productivity and yield are extremely poor. Therefore, it is necessary to engineer acetoin biosynthetic pathway and eliminate competitive pathways for acetoin production. Here, to increase acetoin production, we first eliminated ethanol and glycerol production by deleting alcohol dehydrogenases (ADHs) and glycerol-3-phosphate dehydrogenases (GPDs). By overexpressing α -acetolactate synthase (AlsS) and α -acetolactate decarboxylase (AlsD) from *Bacillus subtilis* in this strain, acetoin and 2,3-butanediol were produced up to 6.0 g/L and 9.5 g/L, respectively. To reduce 2,3-butanediol production, 2,3-butanediol dehydrogenase (Bdh1), which converts acetoin to 2,3-butanediol, was deleted. As a result, 2,3-butanediol accumulation was eliminated and acetoin production was significantly improved up to 15.4 g/L with a yield of 0.30 g/g glucose. Furthermore, by NAD⁺ regeneration through overexpression of water-forming NADH oxidase (NoxE) from *Lactococcus lactis*, the cofactor imbalance generated during the acetoin production from glucose was successfully relieved and acetoin titer and productivity were further increased.

PS6-4: Unraveling the effects of non-*Saccharomyces* yeasts on Sauvignon Blanc aroma profiles; A top-down metabolomics approach complemented by sensory analysis**Margaret Beckner Whitener¹, Jan Stanstrup¹, Valeria Panzeri², Silvia Carlin¹, Benoit Divol², Maret Du Toit², Urska Vrhovsek¹**¹*Fondazione Edmund Mach, Italy;* ²*Institute for Grape and Wine Science, Stellenbosch University, South Africa*

Saccharomyces cerevisiae (SC) is the main driver of alcoholic fermentation but when it comes to aroma and flavor formation in wine non-*Saccharomyces* species can have a powerful effect. This study sought to compare untargeted volatile compound profiles from SPME-GCxGC-TOF-MS and sensory analysis data of Sauvignon blanc wine fermented with six different non-*Saccharomyces* yeasts. The yeasts were allowed to ferment to 2% ethanol; SC was then added to finish the fermentation. The control was SC only. Four of the non-*Saccharomyces* yeasts were commercial starter strains, *Torulaspora delbrueckii* (TD), *Lachancea thermotolerans* (LT), *Pichia kluyveri* (PK) and *Metschnikowia pulcherrima* (MP), while the other two species, *Candida zemplinina* (CZ) and *Kazachstania aerobia* (KA), were isolated from wine grape environments and have shown promise as sensorially beneficial fermentation strains. Each fermentation produced a highly distinct profile both sensorially and chemically. The wines were evaluated for the basic tastes as well as 16 different aromas. Each fermentation was identified as sensorially unique and PCA indicated the dominant flavor and aroma profiles of the yeasts. SC and CZ were the most distinct. SC was characterized by guava, grapefruit, banana, and pineapple aromas while CZ was driven by fermented apple, dried peach/apricot, and stewed fruit as well as sour flavor. Chemically over 300 unique features were identified as significantly different across the fermentations. When clustered hierarchically all of the biological replicates grouped together displaying strain specific profiles. Variances in esters, alcohols and terpenes were the main drivers of chemical differentiation. The SC profile was dominated by esters but all the yeasts had distinct ester profiles likely due to variation in acetyl- and acyltransferase activities between the yeasts. CZ displayed the highest number of terpenes and sesquiterpenes of all the fermentations but also produced a large amount of acetic acid. KA was second highest in terpenes and showed a number of as yet unidentified analytes. TD had few esters but three distinctly higher thiol compounds. The LT showed a relatively high number of ketones and acetate esters. The PK and MP fermentations had high levels of different methylbutyl, methylpropyl, and phenethyl esters. Overall, the sensory and chemistry methods complemented each other well, giving a much more detailed profile of these yeast than anything previously reported.

PS6-5: Impact of unique Lager-specific genes on phenotypes in *Saccharomyces pastorianus***Ursula Bond**, Chandre Monerawela, Ivan Sugrue*School of Genetics and Microbiology, Trinity College Dublin, College Green, Dublin 2, Ireland*

Saccharomyces pastorianus is a recently evolved species used in the brewing of lagers. Two distinct strain types, referred to as Groups I and II, have been identified. The two groups arose by independent hybridisation events between different strains of *S. cerevisiae* and *S. eubayanus*. The group II *S. pastorianus* strains contain four so-called lager-specific genes (TYP, AMD, TRR and HYPO) originating from the *S. cerevisiae* parent. The genes reside in subtelomeric regions and have subsequently been lost, in different combinations, in industrial, wine, laboratory, bakery and bioethanol strains of *S. cerevisiae*. Additionally, the parental genomes have undergone homeologous recombination at specific chromosomal locations, the majority of which lie within open reading frames, creating a unique set of hybrid gene alleles. The presence of unique sub-telomeric genes and hybrid genes has the potential to confer novel phenotypes on the Group II *S. pastorianus* strains. We have characterised several of the lager-specific genes. The gene *HYPO* located at the left sub-telomere of chromosome XVI, encodes a hypothetical opening reading frame with no known prokaryotic or eukaryotic homologues. A GFP fusion of *HYPO* was created and expressed in *S. cerevisiae* strain S-150, which lacks a copy of *HYPO*. The gene was constitutively expressed under fermentation conditions and produced a functional protein that localised to the cell membrane where it accumulated in a patch-like pattern. Surprisingly less than 2% of cells cultured in glucose expressed the GFP fusion. This percentage increased to 10% when cells were cultured in malt-wort. We have also analysed phenotypes conferred by several of the hybrid genes, which exist together with parental genes in different gene copy numbers, in *S. pastorianus* strains. Hybrid alleles complement mutant phenotypes of the *S. cerevisiae* parental gene, however quantitative differences in phenotypes are observed. Since many of the hybrid genes encode proteins required for cell functions such as RNA metabolism, carbohydrate metabolism and storage, and mitochondrial energetics, the global influences of such quantitative differences on the phenotypes of lager yeasts were investigated.

PS6-6: Characterization of *GLT1* and *GDH1* modulation on *Saccharomyces cerevisiae* metabolism and physiology**Marco Brambilla, Giusy M. Adamo, Danilo Porro, Paola Branduardi***University of Milano Bicocca, Italy*

In a biorefinery, different types of biomass are converted into sugars that are used as carbon source by microorganisms, called cell factories, to produce chemicals and biofuels. *Saccharomyces cerevisiae* is one of the most characterized cell factory, employed in different biotechnological applications. Considerable efforts are oriented to the study and the engineering of carbon assimilation by *S. cerevisiae*, in order to achieve a high production, productivity and yield of the bioprocess. However, the study and the eventual engineering of nitrogen assimilation are also of great importance to design cell factories with an improved capability to exploit proteins-rich biomasses in fermentative processes. In *S. cerevisiae*, nitrogen compounds, once inside the cells, are catabolized through the Central Nitrogen Metabolism (CNM), in which the enzymes glutamate dehydrogenase 1 (Gdh1p), glutamate dehydrogenase 2 (Gdh2p), glutamine synthetase (Gln1p) and glutamate synthase (Glt1p) take part. The effects of *GLT1* deletion and over-expression, alone or in combination with *GDH1* deletion, on nitrogen assimilation and cell physiology have been characterized in the presence of different nitrogen sources. Results are shown and discussed, together with future perspective.

PS6-7: Improvement of whole cell transformation of benzaldehyde into (R)-phenylacetylcarbinol by *Saccharomyces cerevisiae***Stefan Bruder¹, Michael Breuer², Eckhard Boles¹**¹*Goethe-Universität Frankfurt, Germany;* ²*BASF SE, Germany*

In the last decades, the production of fine and bulk chemicals by metabolically engineered microorganisms has increased substantially. In particular, pharmaceutically relevant substrates, e.g. drugs or drug precursors with complex structures are increasingly synthesised by biotransformations with tailor-made biocatalysts [1]. Unlike conventional chemical catalysts, the advantages of biocatalysts are reflected by high selectivity, high catalytic

efficiency and mild operational conditions. Unfortunately, these contrast with undesirable side reactions [2]. Already in 1930, Hildebrandt and Klavehn used *Saccharomyces cerevisiae* as catalyst for the production of (R)-phenylacetylcarbinol (PAC), a pharmaceutical precursor of the phenethylamine alkaloid ephedrine and its diastereomer pseudoephedrine [3], which are used for the treatment of acute, allergic as well as vasomotor rhinitis. Despite numerous steps in strain and process development, the major by-product benzyl alcohol plays still a crucial role in this biotransformation. Since one molecule of acetaldehyde derived from glycolysis is fused with benzaldehyde to PAC, an imbalance of NAD+/NADH ratio occurs. The lack of NAD+ is not only compensated by redox processes in the respiratory chain, but also through a reduction of the given substrate benzaldehyde to benzyl alcohol. We could show that beside down-regulation of putative oxidoreductases that mediates these specific activities, global modifications of cofactor balances and ATP consumption could contribute to improve the process. As additionally the stability of the catalyst is influenced by cumulative toxicities of substrate and derivatives, the recycling of the minor by-product benzoic acid through a heterologous pathway turned out to increase the detoxification of the whole process.

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PS6-8: Optimization of environmental parameters for improving recombinant protein production by the host *Komagataella (Pichia) pastoris*

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A number of environmental parameters, including medium composition, aeration, and pH affect the performance of a microbial strain in industrial applications. Thus the task of achieving high productivity by optimising these conditions is challenging since it represents a multidimensional problem and the relative significance of these factors vary substantially in a strain-specific manner. In this study, we have demonstrated how cultivation parameters affect productivity by employing a strain of *Komagataella (Pichia) pastoris* expressing Human lysozyme (HuLy). Our findings suggest that, although the pH of the cultivation is a major determinant of the productivity of the strains expressing HuLy from the inducible *AOX* promoter, this was not the case for the productivity of the strains constitutively expressing HuLy from the GAP promoter. We adopted genetic algorithm (GA) as a search heuristic to achieve an improved array of the environmental factors under investigation. Parameters were varied to evolve the medium composition and culture pH towards our objective of improved productivity. Our results showed that through the use of this evolutionary search algorithm, we achieved an improvement of more than 50 % in lysozyme productivity. The proposed methodology suggests a flexible roadmap for designing an optimized population of dependent system properties to improve processes on a case-specific basis in the domain of recombinant protein production by microbial hosts.

PS6-9: A consensus genome-scale metabolic network reconstruction for *Komagataella (Pichia) pastoris*

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Systems biotechnology integrates omics data with *in silico* modelling to design cells that have improved metabolic properties for industrial applications. For the optimization of recombinant protein production, we need to understand how the metabolic network copes with the burden generated by the production of the foreign protein and what changes in the distribution of fluxes result. Flux Balance Analysis represents a useful approach to trace the re-distribution of fluxes *in silico*, but this is critically dependent on a good predictive model with well-defined metabolic network. The predictive ability of the metabolic network model for *Saccharomyces cerevisiae* has been improved significantly in recent years. However for the industrial yeast, *Komagataella (Pichia) pastoris*, although there are three genome-scale metabolic network models available, they differ in content and use different terminologies to describe the same chemical entities; so making it difficult to compare

them. In this study, we describe our efforts to reconstruct a consensus metabolic network for *K. pastoris*. Since this organism is by far the most commonly used yeast species in the production of recombinant proteins, we believe that the new metabolic model will be of significant in the design of production strains of *K. pastoris*.

PS6-10: “Bug-O-Yeast” – Expression of an insect amylolytic gene in *Yarrowia lipolytica*

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The search for novel enzymes with potential applications in industrial processes is receiving constantly increasing interest of the scientific and industrial community. α -Amylases are one of the most important industrially relevant enzymes, with a number of applications, i.e. baking, brewing, textile and paper industry. One of the most desired traits of α -amylases is their ability to decompose raw, non-pre-treated starch granules. In this study we have cloned α -amylase (Amy1) gene from *Sitophilus oryzae*, and expressed it in a non-conventional yeast species, *Yarrowia lipolytica*. *S. oryzae* is primarily known as a major pest of stored rice, contributing to limitation of food availability for a large number of people. We assumed that high expansiveness of *S. oryzae* in cereal crops may be to some extent attributed to highly active digestive enzymes, especially amylases, synthesized by this insect. *Y. lipolytica* has been selected as a host organism, due to a number of advantageous traits of this species regarding protein expression and secretion. Our results demonstrate that active α -amylase has been secreted to the extracellular space of the transformed *Y. lipolytica* cells, as visualized in starch-plate assay. In a course of preliminary test, we have selected *Y. lipolytica* strain 1.18, exhibiting the highest amylolytic activity, for further studies. Insect α -amylase has been efficiently produced in *Y. lipolytica* 1.18 strain in 5-L bioreactors. The enzyme has been purified from the culture medium through affinity chromatography. Several raw starch species have been tested for digestibility with the insect-derived recombinant α -amylase, produced in recombinant *Y. lipolytica* strain.

PS6-11: Rose-like odor production proteome in *Yarrowia lipolytica* revealed by high-throughput ‘omics’ approach

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Traditionally, highly-graded aroma compounds have been acquired from plant and animal resources or via chemical synthesis from petrochemicals processing. However, high demands of the aroma market lead to gradual exhaustion of some of the natural resources for this application. On the other hand, highly efficient and economical chemical synthesis processes require applying extreme physical conditions, as well as toxic solvents and catalysts, altogether contributing to substantial burden imposed on the natural environment. But the key limitation of chemical synthesis with respect to aromas production is lack of the consumer acceptance. Biotechnology now appears as a powerful alternative in the aroma compounds sector. In our earlier report [1] we have shown that *Y. lipolytica* can produce significant amounts of 2-phenylethanol (2-PE), a chemical compound with pleasant, rose-like scent, mainly generated through L-phenylalanine (L-Phe) degradation pathway (the Ehrlich pathway). Therefore, we have head for identification of molecular identities involved in L-Phe conversion to 2-PE, for potential future modifications. The ultimate objective of the presented study was gaining new knowledge on the molecular bases of this valuable aroma compound synthesis in *Y. lipolytica*. Exploitation of an ‘omic’ approach allowed for careful determination of the key molecular identities that are either directly or indirectly involved in the aroma compounds production, and lead to general assessment of the cell’s physiological condition during 2-PE synthesis. This methodology was considered a strategy of choice, since the Ehrlich pathway is characterized by high level of redundancy. Amongst a number of proteins involved in the amino acid turnover and the central carbon metabolism, enzymes involved in L-Phe conversion to 2-PE have been identified.

[1] Celińska et al. (2013) *J Ind Microbiol Biotechnol.* 40, 389-92

PS6-12: Identification of natural allelic variants underlying nitrogen assimilation differences in a multiparent intercross yeast population

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Most traits, including many oenological phenotypes, are complex and regulated by multiple interacting quantitative trait loci. The first step towards accurate models of trait variability, and a prerequisite for predicting and modulating them, is characterisation of the underlying genetic factors. In this context, nitrogen assimilation preferences during wine fermentation represent a complex trait of industrial interest. The nitrogen assimilation profile of each strain has a great importance on the fermentation kinetics, where low nitrogen concentrations in must can lead to sluggish or stuck fermentations, causing important economic losses. In order to identify allelic variants underlying nitrogen assimilation differences between the main representative *S. cerevisiae* strains, we performed QTL mapping and bulk segregant transcriptome analysis in 169 individuals from the multiparent SGRP-4X mapping population. For this, we estimated nitrogen consumption levels at the end of the fermentation process and performed QTL mapping utilizing nitrogen consumption levels for 14 amino acids and ammonium. Overall, we mapped 27 QTLs for the different nitrogen sources with a stringent LOD score above 8. Among these QTLs we have selected more than 10 candidate genes to validate through a reciprocal hemizygosity approach. In parallel, we also performed a transcriptome analysis on bulks of segregants with extreme nitrogen assimilation profiles for ammonium and glutamine consumption. Altogether, our results extend the currently known catalogue of natural variants underlying nitrogen assimilation differences, representing a useful tool to generate more efficient strains for the wine industry.

PS6-13: Screening of phenolic metabolites from *Saccharomyces boulardii* cell cultures

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Saccharomyces boulardii, a probiotic yeast, has been studied for its antioxidant and immunomodulatory properties. Several secretory factors have also been identified to play vital role in enhancing the therapeutic value of this yeast. However, there is still lack of knowledge in the industrial applications of this yeast. Considering the possibility of detecting industrially relevant biomolecules responsible for its antioxidant properties, a global metabolite screening approach was adopted in this study. We identified the presence of both intracellular and extracellular phenolic metabolites using GC-MS. The metabolite footprints were then assessed by ESI-MS/MS for confirmation. This study was an attempt to initiate a step towards the potential industrial applications of this yeast which can be furthered by large scale recovery of significant metabolites by both upstream/downstream processing methods.

PS6-14: Novel biosensors for accelerating yeast cell factory development

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The development of efficient cell factories is fundamental for establishing a biosustainable economy. Inverse metabolic engineering is used to uncover new targets by high-throughput screening of random genetic libraries. It relies on the selection of a high-producing phenotype from a diverse, previously generated, population of cells. While techniques for generating these diverse cell populations are well established, inverse metabolic engineering suffers from the lack of sufficiently sensitive, selective high-throughput measurement technologies to screen efficiently for high producers. In order to generate a multi-dimensional sensor system, we are engineering new transcription factor based sensors for key metabolites and combine them with fluorescent

protein reporters. We are aiming for optimizing the production of fatty acid derived products ranging from biofuels to food and cosmetic additives. Intracellular biosensors are used to sense limiting key intermediates like malonyl-CoA and acyl-CoA. The used transcription factor based sensors originate from bacterial systems, were codon optimized and heterologously expressed in the yeast system. Genetically diverse libraries were screened for superior acyl-CoA platform strains by Fluorescent Activated Cell Sorting (FACS). The strains were extensively characterized by next generation sequencing. Enriched candidate genes were coexpressed and evaluated on a production pathway for fatty alcohols. The malonyl-CoA sensor system was applied for metabolic pathway control for 3-hydroxypropionic acid (3-HP) production. Auto-induced downregulation of fatty synthesis led to accumulation of the 3-HP precursor malonyl-CoA, which in turn induced expression of the heterologous pathway through dynamic malonyl-CoA sensor control. Introduction of this dual pathway control increased the production of 3-HP by 10-fold and can also be applied for production of other malonyl-CoA derived products.

PS6-15: Heterologous expression of cellulase genes in wild *Saccharomyces cerevisiae*

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Simultaneous saccharification and fermentation (SSF) involves enzymatic hydrolysis of pretreated lignocellulosic biomass and fermentation of its glucose constituents in a single bioreactor. Although *Saccharomyces cerevisiae* is the microorganism of choice for cellulosic bioethanol production, it has a significantly inferior secretory capacity for heterologous proteins compared to other filamentous fungi. Moreover, degradation products resulting from biomass pretreatment impairs growth and fermentation of sugars. One approach to resolve both concerns is to utilize a strain background with innate tolerance to inhibitory degradation products and a high secretory phenotype. In this study, we screened a collection of 32 wild *S. cerevisiae* strains isolated from vineyards in South Africa, with high inhibitor tolerance and superior secretion phenotypes serving as basis for selection. After engineering the strains with high- and low-gene copy expression cassettes harbouring the *Talaromyces emersonii* Cel7A (a cellobiohydrolase), *Trichoderma reesei* Cel5A (an endoglucanase) and *Saccharomyces fibuligera* Cel3A (a β-glucosidase), we compared the expression ability of these engineered strains to the benchmark S288c strain. We also compared fermentation vigor, temperature and osmotolerance. During high copy plasmid expression, engineered strain YI13 showed higher enzyme activity levels and produced a similar amount of ethanol (9,0 g/L) to the benchmark S288c strain. Furthermore, relative to the rest of the evaluated repertoire of strains, this strain exhibited superior tolerance to industrial stresses such as high temperature, hyperosmotic stress and secretion stress. YI13 illustrated high growth vigour, in addition to comparatively higher native invertase secretion. These results validate our pursuit of wild *Saccharomyces cerevisiae* strains with high secretion phenotypes and high tolerance properties for lignocellulosic biofuel production. Some wild yeast strains phenotyped in this work indicate that these hosts have the genetic background that could have potential for fermentation of pretreated biomass.

PS6-16: Fatty acid production in *Saccharomyces cerevisiae* by a disassociated Fatty Acid Synthase system (FAS type II)

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Microbial biosynthesis of fats and oils from renewable carbon sources has attracted significant attention in recent years for the potential production of biofuel and other commodities. Almost all organisms synthesize de-novo fatty acids via a well conserved cyclic series of four reactions involving the condensation, reduction, dehydration and reduction of carbon-carbon bonds. In nature there are two main types of fatty acid synthase systems (FAS), type I and type II. FAS I systems utilize a single large, multifunctional polypeptide and are common to both mammals and fungi, although with some structural differences. On the other hand, Plants and Bacteria's (as well in mitochondria and chloroplasts) utilize the disassociated FAS type II system. The reactions of the type II FAS, unlike FAS I, are catalyzed by discrete and monofunctional enzymes, the proteins are all expressed as individual polypeptides from separated genes. The organization of FAS II enables the synthesis of several fatty acid products and is more amenable to modification of chain length. The separation of functions in single polypeptides also facilitates the metabolic optimization of each reaction step along the sequence, which is

impossible with FASI, since the subunits are stoichiometrically fixed. In this work we implemented a FASII system consisting of 12 individually expressed genes in a *S. cerevisiae* strain carrying a conditional allele of the fatty acid synthase genes of the endogenous FAS system. The physiological consequences of this expression will be discussed. This work was funded by the FCT project MycoFat PTDC/AAC-AMB/120940/2010. F.A. was supported by an FCT fellowship SFRH/BD/80934/2011. This work was supported by FEDER through POFC – COMPETE and by Portuguese funds from FCT through the project PEst-OE/BIA/UI4050/2014

PS6-17: Production of SCO by *Yarrowia lipolytica* on various industrial wastes containing glycerol

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Glycerol is an important renewable feedstock as it is the main side-product of the biodiesel production process, which is nowadays applied on a large commercial scale. Moreover, it is produced by several others industries, such as fat saponification, stearin production and alcoholic beverage production units. Unfortunately, crude glycerol may contain many contaminants that significantly decrease its value. The purification process of crude glycerol is time and energy consuming, as it requires many procedures such as refining through filtration or fractional vacuum distillation. Despite the high contamination, crude glycerol might be easily utilized by yeast *Yarrowia lipolytica*, a well-known oleaginous yeast. *Y. lipolytica* is one of the most extensively studied “non-conventional” yeasts due to its biotechnological potential. This unconventional yeast, which has been classified as a GRAS organism, is able to metabolize this renewable feedstock and has a huge biotechnological potential to produce citric acid and other organic acids, single cell oils (SCO), erythritol, mannitol, or proteins. In our study we used a *Y. lipolytica* A-101 strain, isolated from soil contaminated by gasoline and diesel fuel. To verify the possibility of application of the glycerol containing wastes from various industry branches, we tested a five different types of waste products containing various concentrations of glycerol (42%-87%) as a culture medium components. As a control pure glycerol was used. We tested a lipid content, fatty acid composition and biomass production. To optimize the SCO production based on industrial wastes we also tested optimal glycerol concentration and a range of C/N ratio in producing media. The best results for lipid production were obtained for medium containing glycerol from fat saponification production and reached 1.69 g/L (25% of total cell dry weight). The fatty acid composition of the oil produced by *Y. lipolytica* growing on crude glycerol presented a potential use as biodiesel feedstock, with low PUFA content. Our results suggest that oleaginous yeast *Y. lipolytica* could serve as a good producer of SCO using a waste glycerol generated by biodiesel production and other industries as a low cost substrate. This work was financially supported by the Ministry of Science and Higher Education of Poland - project no. IP2012 008972.

PS6-18: Improved tolerance of *Saccharomyces cerevisiae* overexpressing a mitochondrial Cytochrome C Oxidase gene (COX20) to Hydrogen Peroxide Induced Oxidative stress

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For economical and viable industrial production of bioethanol from lignocellulosic material, microorganisms with a high inhibitor stress tolerance during fermentation are required. *Saccharomyces cerevisiae* is a Crabtree positive yeast which readily converts available monomeric hexose sugars glucose into ethanol and has tolerance towards osmotic and ethanol stress concentrations when compared with other yeast species. Novel yeast strains with inherent properties can be produced by combining selective breeding and genetic engineering to improve industrial production of lignocellulosic bioethanol fermentation. Previously, we identified a cytochrome oxidase gene (*COX20*) from yeast whose overexpression improved tolerance to acetic acid [1]. Presence of acetic acid has been identified as initiating programmed cell death in yeast [2], through a cytochrome C mediated cascade mechanism [3]. Phenotypic microarray (PM) assays with *COX20* deleted yeast strains containing either an empty vector control (pCM173) or a vector designed to express *COX20* (pCM173:COX20) revealed that expression of *COX20* conferred tolerance to hydrogen-peroxide induced oxidative stress. This improved tolerance was confirmed by viability assays, growth on plates and performance in fermentation.

[1] Greetham and Kumar, unpublished; [2] Ludovico P., Madeo F. et al., (2005) *IUBMB* 57, 129.35; [3] Guarangella N., Passarella S. et al. (2011) *Mitochondrion* 11, 987-91

PS6-19: Gene and protein engineering in production of the enzymes of analytical importance

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Analytical biotechnology, including Biosensorics, require very specific biorecognizing elements, among them the enzymes as biocatalytic components are mostly used. To facilitate isolation of the enzymes and their purification and to improve the bioanalytical parameters of the biosensors, gene and protein engineering approaches are very important. For creation of enzyme-based, as well as microbial biosensors, we have constructed recombinant microbial cells overproducing the target enzymes. The high permeability of living cells to nanoparticles was intensively studied in the recent years due to perspectives of such approaches for controllable drug delivery into organism. The gold nanoparticle (nAu) display a unique combination of chemical inertness, surface chemistry, size- and shape-dependent electronic and optical properties, which render them ideal for clinical applications. The recent advancements were reported about the nAu usage in vaccine development, gene therapy, enhanced radiotherapy and others, however, many relevant issues remain open. They include the molecular mechanisms governing the nanoparticles-cell interactions, the physico-chemical parameters underlying their toxicity to different types of cells, the lack of standard methods and materials, and the uncertainty in the definition of general strategies to develop smart drugs and devices based on nanoparticles. The conception of using nanoparticles in biosensor's technology to increase the output is very fresh nowadays. A number of biosensors with immobilized nanoparticles on electrode surface were constructed. On the other hand, recently we have reported a few papers describing improvement of analytical parameters of the microbial sensors by genetic manipulation with the yeast cell. The idea of implementation of exogenous enzyme to increase the amount of the target enzyme in the cells to be used in microbial sensors seems to be not highlighted yet. The enrichment of the sensing cells by the target enzyme has been achieved by a combination of two approaches: 1) on genetic level - by over-expression the corresponding *HpCYB2* gene in the recombinant cells; 2) using nanotechnological approach - by the transfer of flavocytochrome FC *b*₂-bound nanoparticles into the cells. In this report, we present the data confirming that additional enrichment of the recombinant yeast cells by the enzyme bound with nanoparticles improves the analytical parameters of microbial sensor.

PS6-20: Secretome diversity of *Pichia Pastoris* in the presence of different carbon sources

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The methylotrophic yeast *Pichia pastoris* is widely used as host for heterologous protein production and it is also able to use methanol as sole carbon source. Reduced viability in methanol-based expression systems has been reported to be problematic due to the release of intracellular proteases upon cell lysis which is detrimental for the secreted recombinant product. To study this in more detail, we cultivated recombinant *P. pastoris* in a fed-batch process using either glucose or methanol as carbon source. The feed strategy was designed for obtaining equal specific growth rates and biomass development. Product quantity and quality were analyzed and viability of the cultures, host cell lysis and proteolytic activity in culture supernatants were determined. For the analysis of the secretome, proteins in supernatants were identified using LC-ESI/MS/MS. Strikingly, the number of identified proteins was very low in glucose- and also in methanol-grown cultures and the secretomes were comparable. These results indicate that an appropriate process design for recombinant protein production is very important and can prevent extensive product degradation also in methanol-based cultivations thus leading to improved

productivity.

PS6-21: Unraveling the complex trait of ethyl acetate production in *Saccharomyces cerevisiae*

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Volatile esters are key contributors to the aroma in a wide range of alcoholic beverages and impart fruity flavors. Research has shown that the ester imparting banana flavor, isoamyl acetate, is very desirable in high amounts, whereas another ester, ethyl acetate, with descriptors such as "solvent" and "nail polish" negatively affect the flavor profile. To determine the genetic background of the complex trait of ethyl acetate production we used our polygenic platform to identify QTLs (Quantitative Trait Loci) responsible for the production. Firstly, we screened our collection of 500 different strains isolated from beer, wine, spirits production and natural isolates for esters and higher alcohols using gas chromatography with a flame ionization detector (GC/FID). Statistical analysis of the production of ethyl acetate, isoamyl acetate and isoamyl alcohol identified candidates with high and low production of ethyl acetate while keeping an acceptable ratio of isoamyl acetate to isoamyl alcohol to avoid finding an inactive alcohol acetyl-coA transferase gene (*ATF1*). The results show that strong QTLs identified in *ATF1*-deleted pools are masked in the wild-type pools by the effect of an active *ATF1* gene. Responsible genes for the superior flavor ester profile are currently being identified and transferred to commercial beer strains to improve their flavor profile.

PS6-22: Yeast cell factories for production of taxadiene

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Terpenoids are secondary metabolites that have attracted attention as potential pharmaceutical agents. Taxadiene is a diterpenoid which is the key precursor of the potent anti-cancer drug taxol. In order to produce taxol in a more cost-efficient manner, alternative environmentally sustainable and cheaper production routes are needed. Metabolic engineering of microbes such as the yeast *Saccharomyces cerevisiae* offers an alternative solution to Taxol production. Yeast has several advantages over other microbes, including the ability to functionally express eukaryotic P450-dependent enzymes in the pathway from taxadiene to taxol. In yeast, taxadiene can be produced via the Mevalonate Pathway in which isopentyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are used as building blocks for farnesyl diphosphate (FPP) a precursor of taxadiene. In this study we genetically engineered yeast for production of taxadiene. We used various metabolic engineering techniques, including overexpression, deletion or down-regulation of key genes in the MVA pathway to increase flux towards biosynthesis of key intermediates. In addition, heterologous geranylgeranyl diphosphate synthase (GGPPS) and taxadiene synthase (TS) were expressed. We generated several different engineered strains with these alternative designs and the effects of these genetic modifications on taxadiene production were evaluated.

PS6-23: Erythritol production using non-refined glycerol as a carbon source

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Worldwide energy revolution is causing a shortage of some of chemical feedstocks, such as C4 hydrocarbons heavily depends on naphtha-oil. Renewable biomass is a promising alternative resource, even though it is necessary to convert carbohydrates in hydrocarbons using a process such as hydrogenation. However, most biomass consists of polymers comprising C6 sugars; glucose and fructose, and C5 sugars; xylose and arabinose. The biomass widely distributed in nature thus lacks the C4 sugar erythrose, which can be converted to C4 hydrocarbons. Hence, securing a source of a C4 feedstock is an critical issue in the chemical industry. Erythritol (1, 2, 3, 4-butanetetrol) is a rare C4 compound produced by fermentation from glucose. Currently it is used as a low-energy sweetener in food ingredient or moisturizer in cosmetics. Unfortunately erythritol-is considerably

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more expensive than hydrocarbons derived from naphtha-oil, a reduction in cost is critical for its use as a chemical. We found that *Moniliella megachiliensis*, a highly osmo-tolerant yeast, can utilize non-refined glycerol derived from palm oil or beef tallow and efficiently convert it to erythritol. Cell growth in glycerol was almost comparable to in glucose, and the cells could propagate up to 300 mg/mL glycerol. When 200 mg/mL non-refined glycerol was supplied, the yield of erythritol was approximately 60%, much more higher than that obtained using glucose. Globally, the amount of glycerol waste is increasing as the production of bio-diesel increases, so the cost of glycerol might be lower than glucose. Thus, the conversion of glycerol waste into valuable erythritol is attractive and promising from the viewpoint of ensuring a supply of C4 hydrocarbons and utilizing a waste natural resource.

PS6-24: The GLO1 gene is required for the efficient assimilation of sulfate

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Hydrogen sulfide is an off-flavor produced during winemaking and its concentration in finished wine is a critically important parameter determining product acceptability. In a screen of possible carbon-sulfur lyase enzymes, we found that strains of yeast deleted for the GLO1 gene produced elevated H₂S. GLO1 encodes a glyoxylase with a role in detoxifying methylglyoxal that is produced during glycolysis. Strains with deletions in other genes involved in methylglyoxal detoxification showed that the H₂S production was specific to GLO1. Growth measurements established that the glo1 mutant strain assimilated sulfate inefficiently, but grew normally on cysteine. These data are consistent with the idea that the GLO1 protein is required for full activity of the enzyme O-acetyl homoserine sulfhydrylase, encoded by MET17. Overexpression of GLO1 in a wild-type strain replicated both phenotypes of the glo1 deletion mutant: elevated H₂S production and slow growth on limiting sulfate. However, a pull-down experiment could not identify a direct interaction between Glo1p and Met17p.

PS6-25: Engineering yeast cell factories for isoprenoid production

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Isoprenoids comprise a large family of metabolites with various industrially-useful functions including biofuels, flavors, fragrances, nutraceuticals and pharmaceuticals. These metabolites are often produced in nature by organisms that are not optimal for industrial production. For example, many useful isoprenoids that are plant-derived are often found only in low levels in plants and are obtained by complex extraction procedures. Rising demands for such compounds, as well as the environmental impact associated with plant-based extraction creates a need for alternative production routes. Reconstruction of plant biosynthetic pathways for valuable compounds in microbes provides a promising alternative to plant-based production. Particularly, the yeast *Saccharomyces cerevisiae* makes for a very attractive host for such production. It is a well-known and robust industrial organism, has a GRAS (Generally Regarded As Safe) status, is easily manipulated genetically and is able to functionally express eukaryotic cytochrome P450 enzymes. Our group makes use of different metabolic engineering approaches to construct yeast cell factories for production of industrially-relevant isoprenoid compounds. Our most recent advances in this field are presented here.

PS6-26: The role of cytosolic and peroxisomal transaldolase and transketolase in xylose metabolism and alcoholic fermentation in methylotrophic yeast *Hansenula polymorpha*

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The methylotrophic yeast, *Hansenula polymorpha* is one of the known most thermotolerant yeast species with ability to ferment xylose at elevated temperatures (up to 50°C). However, the efficiency of xylose conversion to

ethanol by natural *H. polymorpha* strains is quite low due to complexity of xylose utilization pathway, which includes reactions of pentose phosphate pathway (PPP) and glycolysis. Identification of rate-limiting enzymes for xylose conversion to ethanol is necessary for rational strain modification to improve the fermenting efficiency. Several metabolic engineering approaches have been successfully developed to improve ethanol production from xylose in *H. polymorpha*. However, the role of two key enzymes involved in PPP, namely transketolase and transaldolase, in xylose metabolism and alcoholic fermentation in *H. polymorpha* still remained unknown. The methylotrophic yeast *H. polymorpha* contains in addition to cytosolic transaldolase (gene *TAL1*) and transketolase (gene *TKL1*) also peroxisomal transketolase (also known as dihydroxyacetone synthase, gene *DASI*) and putative peroxisomal transaldolase (gene designated by us as *TAL2*). The knockout or overexpression of these four genes was examined regarding their roles in xylose utilization and fermentation. In the wild type strain of *H. polymorpha* overexpression of *DASI* and *TAL2* genes turned out to be beneficial for xylose alcoholic fermentation. Both knockout strains did not show growth retardation on xylose as carbon source but were impaired in xylose fermentation as compared to the wild type strain. The impact of *TAL1* gene on xylose utilization in *H. polymorpha* seems to be more pronounced as *tal1Δ* mutants were unable to grow on xylose containing medium. The effect of overexpression of *TAL1* and *TKL1* genes on xylose fermentation is under investigation.

PS6-27: Yeast-derived Schmallenberg virus nucleocapsid protein as an antigen to detect anti-viral antibodies in bovine saliva

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Schmallenberg virus (SBV), discovered in continental Europe in late 2011, causes mild clinical signs in adult ruminants, including diarrhoea and reduced milk yield. However, fetal infection can lead to severe malformation in newborn offspring. Enzyme-linked immunosorbent assays (ELISA) are commercially available for detection of SBV-specific antibodies in bovine sera and milk. Here we describe the development and evaluation of indirect ELISA based on the yeast-derived recombinant SBV nucleocapsid protein (N) for the detection of SBV-specific antibodies in bovine saliva. Development of a non-invasive test to detect antibodies in individual bovine saliva samples could potentially provide a test suitable for calves and adult cattle. The aim of this study was to investigate the agreement between the level of antibodies (IgG) measured in milk and sera, and the level of antibodies (IgG and IgA) in saliva. Serum, milk and saliva samples from 56 cows were collected in three dairy herds in Lithuania and tested for the presence of SBV-specific antibodies. The presence of IgG antibodies was tested in parallel serum and milk samples, while the presence of IgA and IgG antibodies was tested in saliva samples. The presence of SBV-specific IgG and IgA in saliva was tested using indirect ELISA based on yeast-derived recombinant N protein. The presence of SBV-specific IgG in milk and sera was tested in parallel by using commercial and recombinant protein based test. The sensitivities of the newly developed tests were as follows: 100% for the IgG serum assay and 100% for the IgG milk assay in comparison with the commercial tests, 85% and 98%, for IgG and IgA in saliva tests, in comparison with commercial serum IgG assay. Data from testing the saliva IgG and IgA and milk and serum IgG with indirect SBV-specific ELISAs showed close agreement with the commercial serum and milk IgG assay data. The level of IgG in saliva was notably lower in comparison to IgA. The newly developed method exhibits the potential to serve as an easily transferable tool for epidemiological studies.

PS6-28:Deregulation of the glycolytic flux at the level of 6-phosphofructo-1-kinase enables fermentative use of some pentose sugars by *Saccharomyces cerevisiae*

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Although all the genes encoding the enzymes needed for the degradation of xylose are present in the wild type *S. cerevisiae*, the yeast can neither ferment nor assimilate pentose sugars. Much has been done in the past to

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engineer yeasts to grow on L-arabinose, D-xlylose or xylitol in order to use pentose sugars for bio-ethanol production. Most genetic modifications were confined to the enzymes of the initial conversion of pentoses to D-xylulose-5-phosphate and the pentose phosphate (PP) pathway enzymes. Yet, no modifications have, so far been described for the improvements of the glycolytic enzymes. We were first to show that the key glycolytic regulatory enzyme 6-phosphofructo-1-kinase (PFK) can be posttranslationally modified to yield a highly active, ATP and citrate resistant shorter fragments that were generated after the proteolytic cleavage of the native enzyme. This event was first described in a commercial microorganism *Aspergillus niger* and later found to be characteristic also for the human cancer cells. Spontaneous posttranslational PFK modification can be avoided by the insertion of the truncated human sf *pfkM* gene that enabled synthesis of the active shorter PFK-M enzymes that deregulated glycolytic flux in the recipient cells. By the insertion of the sf *pfkM* gene into *S. cerevisiae*, unbalanced NADH/NADPH ratio was detected in the transformants. It was alleviated by the insertion of the *maeA* gene encoding NADPH specific cytosolic malic enzyme of bacterial origin. Double sf *pfkM/ maeA* transformants were able to grow on xylitol medium with added 5 mM of glutamic acid under the semi-anaerobic conditions. The wild type yeast strain and the transformant with the gene encoding the native PFK-M enzyme didn't grow in such environment. The strain with the modified PFK-M enzyme grew on xylose, xylitol and maltose but not on arabinose as a single C-source. No ethanol was detected in the medium during the fermentation, although phenyl ethanol was observed to accumulate. These results prove that the deregulation of the glycolytic flux at the level of PFK may be the sufficient for inducing the ability of *S. cerevisiae* to grow on pentose sugars. However, for the improvement of the commercial bio-ethanol producers, the genes enhancing the glycolytic flux should be combined with previously modified genes encoding the heterologous enzymes for the initial degradation of the pentoses and those of the PP pathway.

PS6-29: Genetic breeding of Lager brewer's yeast

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Industrials brewer's yeast strain improvement can lead to a more efficient beer production process, better quality or healthier beer. However, there are major concerns about using genetically modified organisms in the food and beverage industry because of its safety problems, even for self-cloned strains. Lager brewer's yeast (*Saccharomyces pastorianus*), a genetic hybrid and allopolyploid, contributes to 90% of the total beer market. Thus, breeding of brewer's yeast is always a challenge. Traditional genetic approaches have been widely applied in brewer's yeast optimization for better flavor and fermentation performance. With different selection markers, lager yeast strains with lower production of acetaldehyde or diacetyl as well as better flavor were obtained through UV mutation and sophisticated screening methods. Domestication of brewer's yeast in particular medium resulted in corresponding "natural" mutations. With the first genome sequence of a lager brewer's yeast being reported, the omics technologies and inverse metabolic engineering strategies applied to study the detailed genetic background of brewer's yeast.

PS6-30: Influence of the synthesis of glucan in cell wall on yeast autolysis

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Yeast, as an important raw material of beer brewing, is a key factor of influencing the quality of beer. In this study, we overexpressed and knocked out *FKS1* gene, which regulates the synthesis of β-1,3-glucan. The changes of yeast anti-autolytic and anti-stress abilities were observed. The overexpressed strain enhanced the accumulation of β-1,3-glucan in cell wall by 62 % compared with the wild-type. The overexpressed strain increased anti-stress ability at 8% (v/v) alcohol, 50 ng•mL⁻¹ fungus inhibitor, 0.4 mol•L⁻¹ NaCl or starvation stress, which confirmed the potential of strains with more concentration of β-1,3-glucan in cell wall to tackle environment stresses and autolysis in high gravity brewing. Meanwhile, knocking out the corresponding *FKS2* and *FKS3* showed that *FKS2* could make up the deletion of *FKS1*. Furthermore, the expression level of *FKS1* was down-regulated because of the deletion of *FKS3*, which is probably due to the suppressed expression of RPPO gene which was located at upstream of *FKS1* gene.

PS6-31: Hyper-production of Exg1 promotes the biotransformation of mogrosides in *Saccharomyces cerevisiae* kre6Δ mutants

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Bacteria and fungi are able to secrete extracellular enzymes to convert macromolecules into smaller units required for growth and adaptation. Hyper-production of extracellular enzymes is associated with alterations in cell wall structure in fungus. Recently, we identified that *Saccharomyces cerevisiae* kre6Δ mutants can efficiently convert mogroside V into bioactive mogroside IIIE, which has anti-diabetic properties. However, the underlying mechanism is still unclear. In the current study, we examined bioconversion of mogrosides (MGs) by several mutants with defective cell wall structures (such mutants included kre6Δ, kre1Δ, las21Δ, gas1Δ, cwh41Δ, and cwh43Δ); in addition, we examined the cell walls of these mutants by transmission electron microscopy (TEM), zymolyase sensitivity test, and mannoprotein release assay. We found that a group of zymolyase-sensitive mutants, including kre1Δ, las21Δ, gas1Δ, and kre6Δ, exhibited defects in mannoprotein deposition at the outer layer of the cell wall, resulting in leakage of Exg1 enzymes and efficient MG conversion; such defects were not observed in wild-type cells, or cwh41Δ or cwh43Δ mutants. Moreover, kre6Δ mutants released extra mannoprotein into the media, which may be associated with increased aroma and maintenance of the stability of colors in wine. Thus, kre6Δ mutants may be of benefit for the bioconversion of bioactive compounds and the wine making industry in the future.

PS6-32: Improving of stress response and high temperature fermentation efficiency in yeast *Saccharomyces cerevisiae*

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The main way of ethanol deriving is the alcoholic fermentation of sugary substrates by yeast *Saccharomyces cerevisiae*. The hexoses conversion to ethanol by means of glycolysis is an exothermic process which entails the release of energy that is partially released in the form of heat. That is why the fermenters need to be cooled to the optimal temperature conditions (34-35 °C) for yeast during the alcohol fermentation. Cooling of industrial vessels requires significant energy expenditures. Construction of *S. cerevisiae* yeast stains able to efficient alcohol fermentation at temperatures exceeding 35 °C provides advantage during ethanol production by costs reduction for fermenters cooling, and decrease temperature difference for the further distillation. At elevated temperature the productivity of alcohol fermentation increases. Trehalose accumulation and activation of heat shock proteins were shown to be involved in tolerance to elevated temperature in *S. cerevisiae* cells (Wiemken, 1990). Trehalose is synthesized through two sequential steps. Trehalose-6-phosphate synthase (encoded by *S. cerevisiae* gene *TPS1*) catalyzes trehalose-6-phosphate synthesis from glucose derivatives glucose-1-phosphate and UDP-glucose. Trehalose-6-phosphate is further dephosphorylated by trehalose-6-phosphate phosphatase (encoded by gene *TPS2*) to produce trehalose. A gene *HSP104* is a stress tolerance factor that promotes the reactivation of heat-damaged proteins. The vector for multicopy integration was constructed, in which ORFs of *TPS1*, *TPS2* and *HSP104* genes were placed under the control of strong constitutive promoter *ADH1*. The resulting vector was used for transformation *S. cerevisiae* industrial strains. Recombinant strains possessed increased resistance to stress conditions and achieved higher ethanol yields during fermentation at elevated temperature as compared to parental strain.

PS6-33: Transcriptional regulation of *SEF1* gene in flavinogenic yeast *Candida famata*

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Riboflavin is one of the most important vitamins essential for humans and animals. This vitamin is a metabolic precursor of flavin nucleotides, FMN and FAD, involved as coenzymes in numerous enzymatic reactions. This compound is manufactured on a large scale for use in agriculture, medicine and food industry. To date, only one

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relevant regulatory gene, *SEF1*, was found in flavinogenic yeast *C. famata*. Sef1p belongs to the zinc cluster family of proteins. They possess a zinc finger of the Zn (II) 2Cys6-type involved in DNA binding and act as typical transcription factors. To identify the DNA binding sites of Sef1 transcription factor, a one-hybrid system in *Saccharomyces cerevisiae* was used. To this end, the recipient BY4742 strain of *S. cerevisiae* was transformed with 1) a plasmid harboring *C. famata SEF1* gene under the control of galactose-inducible *S. cerevisiae GAL1* promoter and 2) a reporter plasmid bearing *Kluyveromyces lactis LAC4* gene under the control of *RIB1* and *SEF1* promoters of *C. famata*. In addition to the full-length *RIB1* promoter, we also used truncated sequences of *RIB1* promoter, lacking the predicted Sef1 binding sites. β-galactosidase assay served as readout of Sef1 binding to the studied sequences. The developed one-hybrid system proved suitable for studying the interaction of Sef1 with its putative DNA targets. Thus, we show that Sef1 trans-activates *RIB1* transcription in a heterologous host system. Importantly, truncation of *RIB1* promoter substantially decreases its activation. Also, Sef1p directly interacts with its own promoter providing evidence for Sef1 autoregulation. In *C. famata*, riboflavin overproduction depends on concentration of iron (II) ions in the medium. To find out whether *SEF1* transcription is iron-dependent, we used qRT-PCR. For this, total RNA was isolated from *C. famata*, *S. cerevisiae* and *Pichia stipitis* grown in iron-depleted or iron-supplemented conditions. The relative amount of *SEF1* mRNA was calculated using ΔΔCt method with *ACT1* as an internal control. We found that *SEF1* expression in *C. famata* was strongly correlated with iron concentration in medium showing a 3-fold induction in the iron-depleted condition. *P. stipitis*, despite being a non-flavinogenic yeast with lower basal *SEF1* expression than in *C. famata*, displayed similar trends, suggesting conserved *SEF1* functions in these two species of CTG clade. Unlike, iron transcriptional regulation of *SEF1* was not observed in *S. cerevisiae*.

PS6-34: mRME (mRNA metabolic engineering): a novel approach to obtain industrial phenotypes

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Biotechnological processes are of increasing significance for industrial production of fine and bulk chemicals, including biofuels. Under operative conditions microorganisms meet multiple stresses such as non optimal pH, temperature, oxygenation and osmotic stress. Moreover, they have to face inhibitory compounds released during the pre-treatment of lignocellulosic biomasses, which constitute the preferential substrate for second generation production processes. All together these factors impair cellular metabolism and growth and, as a consequence, reduce the productivity of the process. The highly desirable evolution of robust cell factories is rarely ascribable to a single molecular element, since it requires a complex cellular reprogramming, implying the simultaneous modification of many regulatory and operative elements. In addition to transcription, cells can modulate their complex phenotype by controlling mRNA metabolism and trafficking, translation and finally post-translational modifications. During stressful conditions the translational machinery slows down and the mRNAs are aggregated in cytoplasmatic ribonucleic foci, known as stress granules, where transcripts are stored until the cell has been adapted to the stress. Poly(A) tail length represents an important threshold for mRNAs rate of degradation or translation. Pab1 is the yeast major poly(A) binding protein, playing an important role in mRNA metabolism modulation, and it is also a known component of stress granules. Here we present our approach for manipulating post-transcriptional events in the yeast cell factory *Saccharomyces cerevisiae* by modulating and mutagenizing *PAB1* as a key regulatory element.

PS6-35: Design of a lignin-cellulosome on *Saccharomyces cerevisiae* cell surface for consolidated bioprocessing

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One of the major obstacle for the production of lignocellulosic bioethanol at large-scale is the absence of low-cost technology to break down cellulose into fermentable carbohydrates. Consolidated bioprocessing (CBP), which combines enzymes production, cellulose hydrolysis and sugar fermentation in a single step, is a promising strategy for ethanol production with costs reduction. However, in nature there is not one microorganism able to perform the complete biotransformation of lignocellulosic sugars into ethanol. In the last years, several groups reported the direct conversion of cellulose into ethanol by *Saccharomyces cerevisiae* strains engineered to

display on the cell surface an artificial mini-cellulosome composed of cellulolytic enzymes. However, the levels of ethanol produced are low, suggesting that an improvement of enzyme activity is necessary to exploit cellulosome displaying *S. cerevisiae* as a CBP platform organism. Here, we describe the concept of a novel type of cellulosome, named “lignin-cellulosome”, in which cellulolytic enzymes are assembled on *S. cerevisiae* cell surface together with lignin modifying enzymes (LMEs). In this system, lignin degradation performed by LMEs should reduce the negative effect of lignin on cellulases activity, which occurs during enzymatic hydrolysis of lignocellulose. Preliminary data about the recombinant expression of some proteins intended to be used in this system will be shown and commented.

PS6-36: Identification by QTL mapping of new genes and their allelic forms conferring robustness to environmental variations in wine fermentation

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Quantitative genetics studies the effect of natural variations that contributes to shape complex trait variation. Over the last decade *S. cerevisiae* has rose as a gold model for deciphering the genetic architecture of complex traits and many studies have been carried out for identifying natural variations explaining phenotype differences [1]. In the biotechnological field, these approaches are particularly relevant because they offer the possibility to unearth new alleles from industrial strains. Applying different cross designs (F1-hybrid or backcrosses) and using complementary genotyping methods (PCR-genotyping, DNA-microarrays, SNP calling with NGS) we identified several natural variations explaining the technological performance of industrial wine yeast strains. Once identified, these QTL were “molecularly dissected” at gene or nucleotide level allowing the identification of causative mutations. Our results shed light on the role of new alleles in the *S. cerevisiae* genes *SSU1*, *OYE2*, *VHS1*, *SEP7*, *WHI4*, *PMA1* and *VMA13* regarding relevant traits for industry such lag phase [2], temperature resistance [3], cell size and pH homeostasis [5]. Some of the causative mutations identified are wide spread among wine yeast population while others are rare mutations conferring a positive adaptation to specific conditions. Interestingly, according to their allelic forms, all these genes showed significant interaction with wine conditions (sulfite concentration, temperature, nitrogen content, pH) underlining the major impact of GxE interactions in complex traits determinism.

[1] Liti G. and Louis E. (2012) *PLoS Genet.* 8, e1002912; [2] Zimmer B.L. et al. (2014) *Plos One* 9, e108541; [3] Marullo et al. Submitted; [4] Marti Raga et al. *in prep.*

PS6-37: Indigenous yeasts in the wine: exploring their phenotypic and metabolic biodiversity

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Winemaking is a highly industrialized process and a small number of commercial *Saccharomyces cerevisiae* strains are used around the world, neglecting the diversity of native yeast strains that are responsible for the production of wines peculiar flavours. The aim of this study was to exploit the natural phenotypic richness of fermentative yeasts from the vineyards, to establish the *S. cerevisiae* volatile metabolome and to assess inter-strains variability. To fulfill this objectives 5115 *S. cerevisiae* were isolated from spontaneous fermentations of 13 different grape varieties, and were genotyped and grouped in 1436 strains. A phenotypic analysis of a subset of these strains uncovered a highly variable set of phenotypes and the most variable were the tolerance to 42°C, the sensitivity to metallic ions used in vineyard treatments, drug resistance and the adaptability to nutrient depletion. The fermentative potential of selected strains was evaluated on small-scale fermentations at a partner's

winery by inoculation of musts with each strain. The fermentation of the musts inoculated with endogenous strains showed similar fermentation kinetics to the commercial strain. The volatile profile of two indigenous and two commercial *S. cerevisiae* strains was analysed using a methodology based on advanced multidimensional gas chromatography (HS-SPME/GC×GC-ToFMS) tandem with multivariate analysis. A total of 257 volatile metabolites were tentatively identified, distributed over the chemical families of acetals, acids, alcohols, aldehydes, ketones, terpenic compounds, esters, ethers, furan-type compounds, hydrocarbons, pyrans, pyrazines and S-compounds. Principal Component Analysis (PCA) was used with a dataset comprising all variables (257 volatile components), and a distinction was observed between commercial and indigenous strains, which suggests inter-strains variability. In a second step, a subset containing only esters and terpenic compounds (C_{10} and C_{15}), metabolites of particular relevance to wine aroma, was analysed also using PCA. The terpenic and ester profiles express the strains variability and their potential contribution to the wine aromas, specially the BT2453, which produced the higher terpenic content. This phenotypic and metabolomic research contributes to understand the diversity of indigenous wine microflora versus commercial strains and achieved knowledge that may be further exploited to produce wines with peculiar aroma properties.

PS6-38: Low cytosolic pH signals growth reduction and stress tolerance enhancement

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In the process of bioethanol production yeast biomass is recycled using treatment with dilute sulfuric acid to control the bacterial population. This treatment can lead to loss of cell viability, with consequences on the fermentation yield. To understand and ideally enhance yeast cell survival in these conditions, we analyzed the functional cellular responses to inorganic acid stress. Mutants in cell wall integrity (CWI) and Ca^{2+} signaling have reduced growth and viability at a low external pH (pH_{ex}) of 2.5, whereas WT yeast loses viability only at a pH_{ex} of 1.5. This shows that functional cell wall biogenesis is crucial for survival at low pH. To our surprise, the CWI and Ca^{2+} signaling mutants showed *enhanced* viability at a pH_{ex} of 1.5 compared to pH_{ex} 2.5. To understand this, we analyzed growth and intracellular pH (pH_i) in these conditions, using the pH-sensitive GFP derivative ratiometric pHluorin expressed in the cytoplasm of the strains. While at a pH_{ex} of 2.5 the pH_i was unaffected compared to pH_{ex} 5.0, at pH_{ex} 1.5 the pH_i of a CWI mutant was reduced. This reduction by itself did not lead to loss of viability. Rather, *induced* reduction of pH_i at pH_{ex} 2.5 *rescued* the CWI and Ca^{2+} signaling mutants. We next determined that the lethal effect of low pH_{ex} on CWI and Ca^{2+} signaling mutants take place only in growing cells. We propose that low *extracellular* pH leads to cell wall damage, killing growing yeast that cannot properly repair their wall. However, a low *intracellular* pH reduces growth rate, redirects resources to enhance robustness, and thus protects the cells from death. Likely, yeast has evolved such mechanisms in its ecological niche, where a low pH_{ex} occurs in conditions of weak organic acid presence (such as fruit juice or wine fermentations), and therefore always leads to a simultaneous pH_i reduction. Cytosolic pH emerges as a signal that directs the growth-stress tolerance trade-off in *S. cerevisiae*.

PS6-39: A novel two-stage fermentation process of erythritol production by yeast *Y. lipolytica* from molasses

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Yarrowia lipolytica is a proper host for erythritol production. In present study, a novel two-stage fermentation process of erythritol production based on molasses and glycerol was investigated. First, the biomass of *Y. lipolytica* was grown on medium containing sucrose as a sole carbon source. Next, after utilization of the sucrose by yeasts, glycerol was added into medium. In order to use molasses as a substrate for erythritol synthesis, sucrose utilization was established by expressing the *Saccharomyces cerevisiae* *SUC2* gene. Molasses, as a main by-product of sugar industry is a suitable substrate for high biomass production. Glycerol is also the by-product

derived from the diesel production process, moreover it was shown, as a crucial factor for an efficient erythritol synthesis by *Y. lipolytica*. In our study, during the cultivation yeast *Y. lipolytica* was able to produce 52-142 g l⁻¹ of erythritol, with the productivity oscillated from 0.3 to 0.85 g l h⁻¹ and yield 0.24-0.72 g g⁻¹. During these processes, the biomasses oscillated from 25 to 41 g l⁻¹. This work presents genetically modified strains of *Y. lipolytica* as suitable tools for direct conversion of industrial molasses and glycerol into value added product erythritol. This work was financed by the polish National Centre for Research and Development under Project LIDER/010/207/L-5/13/NCBR/2014 “Improving the biosynthesis of natural sweeteners from renewable feedstock by the yeast *Yarrowia lipolytica*”.

PS6-40: Metabolic engineering of the yeast *Saccharomyces cerevisiae* toward increase of glycerol production

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Glycerol (1,2,3-propanetriol) has multipurpose uses. Currently, it is mainly recovered as a by-product of biodiesel and soap manufacturing or produced from propylene and allyl alcohol. Alternatively, glycerol can be produced by microbial fermentation, using sustainable carbohydrate feedstocks. There is continuous interest in development of the improved microbial strains which effectively convert cheap feedstocks (e.g. glucose) to glycerol. Very efficient processes of microbial glycerol synthesis based on the use of osmotolerant yeasts, algae and some bacteria are known, however, all they are based on aerobic organisms, so glycerol production demands air or oxygen purging which considerably increases production costs. Therefore the development of the *Saccharomyces cerevisiae* yeast strains capable of efficient glycerol production from glucose under anaerobic conditions is of great interest. Yeast *S. cerevisiae* is able to produce substantial amounts of glycerol after adding sulfites, which traps acetaldehyde, to the medium or during fermentation at alkaline pH. However, glycerol yields are low and in the case of sulfite process, operation with large amounts of aldehyde-bisulfite adducts causes environmental concerns. Much more efficient would be the metabolic engineering of *S. cerevisiae* strains producing glycerol as main product under anaerobic conditions. Native mitochondrial acetolactate synthase (Ilv2) catalyzes conversion of pyruvate to acetolactate. It was assumed that cytosolic activity of Ilv2 could decrease intracellular pyruvate concentration and as a result increase glycerol production. Truncated version of Ilv2 lacking mitochondrial targeting signal was overproduced in *S. cerevisiae*. Constructed strain possessed elevated Ilv2 activity and increased glycerol production. In *S. cerevisiae* glycerol is synthesizes from dihydroxyacetone phosphate by subsequent action of by glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Multicopy integration module was used for expression of hybrid gene *GPD1-GPP2* encoding artificial fusion of Gpd1 and Gpp2 under the control of strong constitutive promoter of the alcohol dehydrogenase gene on the background of strain overproducing cytosolic Ilv2. Glycerol production of the constructed strain was 4-fold increased as compared to the parental strain.

PS6-41: Fermentation ability of bottom fermenting yeast exhibiting defective entry into the quiescent state

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In beer brewing, the yeast plays an important role not only in the alcohol production but also in the flavor profiles of beer. Therefore, the fermentation ability of brewing yeast affects on the quality of beer products. The fermentation ability of brewer's yeast has been studied for a long time. Recently, it was reported that the elevated fermentation ability of *Saccharomyces cerevisiae* yeast used for Japanese sake brewing is related to the defective transition into the G0 phase (quiescent state) in cell cycle. To investigate the relationship between the G0 entry and the fermentation ability of bottom fermenting yeast, we constructed two genetically modified strains of Weihenstephan34/70. One is *S. cerevisiae* type RIM15 gene disrupted strain, the other is *S. cerevisiae* type

CLN3-1 mutant strain. Both strains exhibited the phenotypic properties, characterized by the defective entry into the quiescent state. As a result of the fermentation test in the synthetic medium, it was revealed that the fermentation abilities of the constructed strains, such as the sugar utilization efficiency, were enhanced compared to those of the wild-type strains. These results suggest that there is a relationship between the fermentation ability and cell cycle in bottom fermenting yeast and that the manipulation of the relevant genes leads to the construction of yeast strains with higher fermentation ability. This is the first report that indicates the defective G0 entry may induce the modified fermentation profiles of bottom fermenting yeast.

PS6-42: Metabolic engineering for improved production of isobutanol via increasing mitochondrial pyruvate pool in *Saccharomyces cerevisiae*

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Higher alcohols including isobutanol are considered as next generation transport fuels because of their higher energy density and lower moisture absorption compared with ethanol. *Saccharomyces cerevisiae*, which produces small amounts of isobutanol via Ehrlich pathway from the catabolism of valine, is a promising host for the production of isobutanol because of its high tolerance to alcohols and other harsh industrial conditions. In this study, isobutanol was produced in mitochondria by targeting two cytosolic enzymes of the Ehrlich pathway (Aro10 and Adh2) to the mitochondria and increasing mitochondrial pyruvate pool. First, *ALD6* encoding aldehyde dehydrogenase and *BAT1* involved in valine synthesis were deleted to eliminate competing pathways. In addition, genes involved in isobutanol production (*ILV2*, *ILV3*, and *ILV5*), *LEU3Δ601* which encodes a constitutively active form of Leu3 transcriptional activator for leucine biosynthesis, as well as *ARO10* and *ADH2* containing mitochondrial targeting sequences were overexpressed. To increase pyruvate flux to isobutanol synthesis in mitochondria, *LPDI*, one of component of pyruvate dehydrogenase complex, was deleted. Furthermore, mitochondrial pyruvate carrier (MPC) proteins were overexpressed to enhance the mitochondrial pyruvate pool. Among the combinatorial overexpression of three MPC proteins, Mpc1, Mpc2, and Mpc3, forming a hetero-oligomeric complex in the inner mitochondrial membrane, overexpression of *MPC1* and *MPC3* was most effective in increasing isobutanol production. The final engineered strain produced 272.69 mg/L isobutanol from 20 g/L glucose, exhibiting a 18-fold increase in production compared with wild type.

PS6-43: Development of the *Hansenula polymorpha* strains-overproducers of recombinant yeast arginase Car1

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Tumor cells often exhibit an elevated requirement for amino acid arginine and artificial enzymatic arginine restriction has been studied for many years as a mean to induce the death of malignant cells. Normal cells deprived for arginine are able to arrest their cell cycle and enter into a quiescent state from which they efficiently recover following the reintroduction of arginine. The use of recombinant human arginase therapy to restrict arginine bioavailability for selectively targeting malignant cells is currently under clinical trials. To construct yeast strains suitable for arginase overproduction, we isolated *Hansenula polymorpha* mutant deleted in the *HpCAR1* gene encoding for a single yeast arginase (identified in *H. polymorpha* genome database by high sequence homology to baker's yeast *Saccharomyces cerevisiae* arginase gene *CAR1* and to two human arginases' sequences). Arginine-non-utilizing phenotype of the isolated *H. polymorpha* Δ *car1* strain confirmed that, as predicted, and similarly to *S. cerevisiae* counterpart, the deleted gene encodes the main arginine-degrading enzyme in this yeast. This strain was subsequently utilized for construction of *H. polymorpha* intracellular overproducers of native arginase *Car1*. We isolated *CAR1* ORF by PCR using genomic DNA of *H. polymorpha* strain NCYC495 as a template and subcloned it under control of constitutive promoter of *H. polymorpha* glyceraldehydes-3-phosphate dehydrogenase (GAP). Enzymatic activity and arginase protein level were assayed in transformant strains. We observed that, as expected, Δ *car1* strain exhibited no measurable enzyme's activity, arginase specific activity in best strains-overproducers exceeded that in the wild-type strain 75 fold and was not dependent on the presence of arginine as an inducer in the culture medium. Therefore, arginase overproduction is

not toxic or detrimental for the yeast host. We also constructed gene expression vector for secretory form of yeast arginase CarI tagged with HIS6 sequence for efficient affinity purification. The strain Rb11ura3 has been transformed with the vector carrying the modified CAR1 gene under formate dehydrogenase FMD promotor and URA3 gene as selectable marker, and multiple copy integrants have been isolated. The cultivation conditions were optimized for the maximal recombinant product yield.

PS6-44: The yeast *P. pastoris* as a tool for large scale production of WT and mutant human amino acid transporter SLC1A5

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Heterologous expression of human proteins is a useful strategy for producing proteins in large scale for structural and functional analyses. However, in the case of membrane transporters, heterologous expression is very often challenged by the hydrophobic properties of these proteins besides by the different codon usage with respect to those of microorganisms. Amino acid membrane transporters such as B⁰AT1 and ASCT2 revealed particularly toxic for bacteria and, therefore, it was nearly impossible to express them in *E. coli*. To overcome this difficulty yeast was employed. *S. cerevisiae* expressed ASCT2 with very low yield. Therefore, *P. pastoris* was employed, that was shown to be a good alternative in terms of efficient over-expression. cDNA of ASCT2 containing a C-terminal 6-His fusion tag was cloned into a pPICZB vector to give the construct X33/pPICZB-(wt)hASCT2-6-His used for transformation of the yeast strain X33. To improve expression level hASCT2 gene was codon optimized for *P. pastoris* by GenScript and the artificial cDNA included a 5' EcoRI restriction site plus the Kozak consensus sequence and a 3' XbaI restriction site plus a C-terminal 6-His fusion tag. In the optimized gene, the Codon Adaptation Index (CAI) was upgraded from 0.51 (wild type) to 0.82 (optimized) and the GC content was decreased from 63 % to 45 %. A more than two-fold yield of (Opt)hASCT2 was reached as compared to the (wt)hASCT2 (10 mg/L of cell culture). The over-expressed hASCT2 reaches yeast membrane from where it is extracted by C12E8 in a native form. A purification protocol on Ni-NTA resin has been pointed out exploiting the presence of the His-tag. The extracted hASCT2 is not glycosylated by *P. pastoris*; indeed its apparent molecular mass corresponded to that of hASCT2 extracted from HeLa cells after extensive treatment with glycosidase. The hASCT2 transport function was assayed in proteoliposomes. It mediated a sodium dependent antiport of external [³H]glutamine with internal glutamine. No functional difference was found between the recombinant protein and that extracted from HeLa cells, indicating that glycosylation does not influence activity. Finally, *P. pastoris* allowed the over-expression of hASCT2 mutants in which each of the single Cys residues has been substituted by Ala. The mutant cDNA constructs were obtained by the PCR overlap extension method. After insertion in the same plasmid of WT, the mutants were over-expressed in *P. pastoris*.

PS6-45: Modular pathway rewiring enables L-ornithine production in engineered yeast

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Baker's yeast *Saccharomyces cerevisiae* is an attractive cell factory for production of chemicals and biofuels. Many different products have been produced in this cell factory by reconstruction of heterologous biosynthetic pathways, but endogenous metabolism by itself involves many metabolites of industrial interest and deregulation of endogenous pathways to ensure efficient carbon channelling to such metabolites is therefore of high interest. Furthermore, many of these may serve as precursors for biosynthesis of complex natural products and hence development of strains over-producing pathway intermediates can serve as platform cell factories for production of such products. Here, we implemented a Modular Pathway Rewiring (MPR) strategy and demonstrated its use for pathway optimization resulting in high-level production of L-ornithine, an intermediate

of L-arginine biosynthesis and a precursor metabolite for a range of different natural products. Our study represents the first comprehensive study on over-producing an amino acid intermediate in yeast, and our results demonstrate the potential to use yeast more extensively for low-cost production of many high-value amino acid-derived chemicals.

PS6-46: Phenotypic landscape of non-conventional yeast species on different desirable traits for bioethanol fermentation

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Non-conventional yeasts present a huge, yet barely exploited, resource of yeast biodiversity. Many of these yeast species exhibit industrially relevant traits such as ability to utilize complex substrates as nutrients, extreme tolerance against environmental stress and fermentation inhibitors. Prior to this study, high throughput phenotypic evaluation has been performed on a yeast culture collection, consisting of 225 different strains from 81 different non *saccharomyces* species, on desirable stress tolerance traits for bioethanol production. This allowed the identification of 12 multitolerant yeast strains that belong to 12 species and representing 4 families and 8 different genera. These selected strains were subjected to small scale semi anaerobic batch fermentations (100ml) to evaluate the efficiency of fermentation by measuring sugar consumption and ethanol production. In optimal conditions (10% glucose +YP in 30°C) all but one strains, successfully finished fermentation. This is followed by separate fermentation with these selected strains in stressful conditions relevant to bioethanol production such as high temperature (40°C), high osmotic pressure (10% glucose + 50% sorbitol), 0,8% of acetic acid, 3g/l of HMF. Performances of the selected strains were compared to two widely used industrial bioethanol producing *S. cerevisiae* strains (CAT1 and Ethanol Red). Finally, 5 strains with desirable fermentation characteristics were subjected to fermentation with lignocellulose hydrolysate. Our results revealed the phenotypic landscape of several non-conventional yeast species which have not been previously characterized on stresses relevant for bioethanol production. It has identified several extremely tolerant non-*saccharomyces* yeasts for each stress conditions evaluated and opened the possibility to further investigate these species to harness the molecular basis of their extreme tolerance traits to improve the 1st and 2nd generation bioethanol fermentation. Moreover, the results showed that some non-conventional yeast species have comparable or even better fermentation efficiency to *S. cerevisiae* in presence of certain stressors. Together, we presented the potentiality of non-*saccharomyces* to emerge as an alternative solution for bioethanol fermentation.

PS6-47: Conversion of coproducts - Molasses and glycerol into erythritol in two-stage process by engineered strains of *Yarrowia Lipolytica* with invertase

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Yarrowia lipolytica has natural ability to grow on fats or paraffins however it metabolizes only few sugars, ie. glucose, fructose and mannose. This species does not have the ability to hydrolyze sucrose – main component of molasses. In this work the transformants of *Y. lipolytica* carrying invertase from the yeast *Saccharomyces cerevisiae*, were used for efficient production of erythritol in two-stage process. In a biomass production step molasses was used as a carbon source followed by erythritol biosynthesis from glycerol, another cheap substrate. Glycerol was added after 24 h of the culture. One of the tested strains – SUC 2/6 produced 107.4 g l⁻¹ of erythritol, with a yield 0.54 g g⁻¹ from the consumed substrate and a productivity of 0.96 g l⁻¹ h⁻¹. The biomass concentration reached 34.5 g l⁻¹. These results demonstrates the usefulness of molasses and glycerol, two renewable raw materials, for efficient erythritol biosynthesis in two-stage process. This work was financed by the National Centre for Research and Development under Project LIDER/ 010/207/L-5/13/NCBR/2014

"Improving the biosynthesis of natural sweeteners from renewable feedstock by the yeast *Yarrowia lipolytica*".

PS6-48: Metabolosomes: construction of synthetic organelles in *Saccharomyces cerevisiae*

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Compartmentalization of metabolic pathways in membrane-surrounded organelles is a powerful tool to overcome several obstacles associated with the engineering of metabolic pathways, such as unwanted side reactions, accumulation of toxic intermediates, drain of intermediates out of the cell, and high diffusion distances. Strategies employing natural organelles such as mitochondria suffer from the presence of endogenous pathways and transporters. In our approach, we make use of endoplasmic reticulum-derived vesicles ('metabolosomes') generated by the overexpression of synthetic peptides containing the N-terminal proline-rich and self-assembling region of the maize storage protein gamma-Zein ('Zera') fused to model enzymes. Transport of substrates and products in and out of the metabolosomes is achieved by targeting of transporters to the ER-membrane, allowing the incorporation of these transporters into the metabolosome membrane. Using fluorescence microscopy and cell fractionation techniques, we have proven the formation of metabolosomes and the cosedimentation of transporters and model Zera-fusion enzymes in membrane fractions. Enzyme activity in the membrane fractions could be verified by enzyme tests. As a proof of principle, we performed growth tests with Zera-β-galactosidase, Lac12p and Gal2p expressing *Saccharomyces cerevisiae*. Our results indicate the formation of functional, lactose-utilizing metabolosomes containing β-galactosidase and both sugar transporters.

PS6-49: *CAT8* gene is involved in regulation of xylose alcoholic fermentation in the thermotolerant methylotrophic yeast *Hansenula polymorpha*

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Xylose is considered as semi-fermentative carbon source showing features of both fermentative and respiratory substrates. Being important carbon source for alcoholic fermentation, search for approaches which activate fermentative abilities of this pentose and simultaneously block its respiration is of great interest. We pay attention to *CAT8*, the global transcriptional regulator involved in regulation of gluconeogenesis and utilization of alternative to glucose carbon sources in *Saccharomyces cerevisiae*. However, the functions of *CAT8* homologue in the thermotolerant methylotrophic yeast *Hansenula polymorpha* were not studied. During last decade, strains of *H. polymorpha*, which is promising organism for high-temperature alcoholic fermentation of lignocellulosic sugars, accumulating 15-20 times more ethanol from xylose have been constructed, however, parameters of xylose alcoholic fermentation have to be further improved to meet requirements for feasible cost-effective process. Homologue of *S. cerevisiae* *CAT8* gene was isolated from the sequenced strain *H. polymorpha* NCYC495 and used for construction of the deletion cassette. The strains with knock out in *CAT8* gene were constructed on the background of the wild-type strain and available the best ethanol producer from xylose. Both types of deletion strains have defect in growth on gluconeogenic substrates (glycerol, ethanol) whereas growth on glucose and xylose was not affected. The mutants *Δcat8* isolated from the wild-type strain did not show changes in ethanol production in glucose whereas accumulated 2-3 times more ethanol in the medium with xylose. The *Δcat8* mutants isolated from the most advanced ethanol producer from xylose also did not show any differences in ethanol production in glucose whereas accumulated 25-30% more ethanol in the medium with xylose. Maximal accumulation from xylose reached 12.5 g/L of ethanol at 45°C which exceeds ethanol accumulation in the wild-type strain NCYC495 near 25 times. Data on the expression of genes involved in xylose metabolism, glycolysis, gluconeogenesis, pentose phosphate pathway and respiration as well as on the specific activities of the corresponding enzymes will be provided. Summarizing, it could be concluded that the transcription regulator *CAT8* is apparently involved in repression of xylose alcoholic fermentation and consequently its damage strongly activates this process and could be useful for construction of the industrial xylose fermenting strains.

PS6-50: Efficient phosphate recovery from agro waste streams by enzyme, strain, and process engineering

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Phosphate stewardship and ultimately recycling is one of the great challenges of humankind. Against this backdrop we propose a new value chain to recover phosphate from plant waste material and to convert it to polyphosphates of industrial value. The approach is based on naturally occurring enzymes that free the phosphate bound in an organic form (mainly phytate in oilseeds) and microbes that effectively collect the soluble phosphate to store it as polyphosphate. These microbial super collectors will be harvested and the synthesized polyphosphate recovered. Thereby, the currently implemented value chain of phosphate rock mining, production of phosphoric acid, chemical synthesis of polyphosphates, and after use phosphate disposal into waste water, rivers and finally into the ocean will be extended and in the long run disrupted.

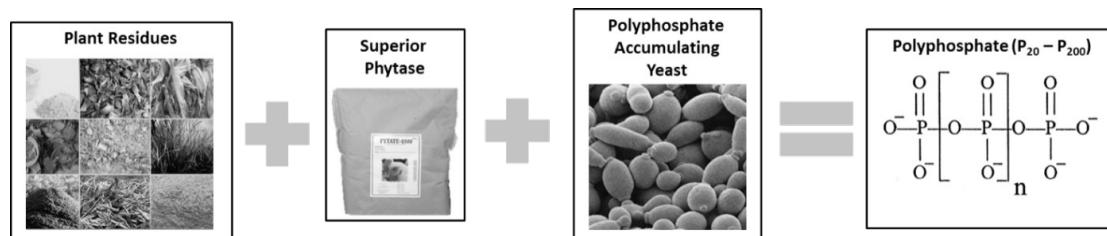


Figure: Proposed approach of phosphate stewardship: production of high-molecular weight polyphosphate with designer phytases and phosphate collecting yeast.

Phytases are extremely highly active phosphatases (>1000 U/mg), mobilizing inorganic phosphate from plant based phytate, which is a natural plant phosphate reservoir, e.g., 72% of phosphate content in corn is bound in phytate [1]. We aim in the project for improving specific activity and thermal resistance of a selected phytase by directed evolution. Tailoring catalysts properties by directed evolution to specific application demands in terms of stability, enantioselectivity or stereoselectivity, became a standard approach in biocatalysis, medical science and synthetic biology [2]. The core expertise of the institute of biotechnology is the rational and evolutive design of proteins [3,4]. Projects range from fundamental science to understand structure-function relationships over protein modeling to methods development for directed evolution and optimization of biocatalysts for sustainable production from renewable resources. Acknowledgement: This work is part of the P-ENG project, funded by the “NRW-Strategieprojekt BioSC”.

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PS6-51: Construction and evaluation of recombinant strains *Saccharomyces cerevisiae* with deletion of *ADH1*, *ADH2* genes and overexpression of *GPD1*, *GPP2* genes for improvement of glycerol production

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Glycerol is widely used in cosmetical, food, tobacco, pharmaceutical, leather and textile industries. In addition, it is considered as a cheap raw material for microbial fermentation. That is why the construction of yeast strains-producers of glycerol became an actual objective for modern metabolic engineering. Our strategy comprised the deletion of *ADH1* and *ADH2* genes, encoding alcohol dehydrogenase in *S. cerevisiae* and overexpression of both glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase genes (*GPD1*, *GPP2*) in $\Delta adh1$ strain in order to redirect the glycolytic pathway to a glycerol synthesis. For this purpose, *S. cerevisiae* strains

with double deletion of *ADH1* and *ADH2* genes and *Δadh1* strain with simultaneous overexpression of *GPD1* and *GPP2* genes were constructed. Obtained mutants were characterized biochemically. Alcohol dehydrogenase activity of recombinant *Δadh1Δadh2* strain was approximately 1.5 fold lower than that in *Δadh1* or *Δadh1-GPDGPP_11* strains. This confirmed *ADH2* deletion on the *Δadh1* background. Glycerol yield during the course of fermentation was increased by approximately 10% in a double deletion strain compared to *Δadh1* (10.6 g/L) reaching 11.9 g/L on the third day of fermentation. Biomass accumulation during the whole course of fermentation (72 hours) was slightly decreased in a double deletion mutant compared to *Δadh1* and *Δadh1-GPDGPP_11* strains. Recombinant *Δadh1* and *Δadh1-GPDGPP_11* strains consumed less amounts of glucose than the parental strain. Furthermore, a double deletion mutant consumed slightly less amounts of glucose compared to *Δadh1* strain during the course of fermentation.

PS6-52: Construction of yeast *Saccharomyces cerevisiae* recombinant strains with increased glycerol production under anaerobic conditions

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Glycerol is used in cosmetic, paint, automotive, food, tobacco, pharmaceutical industries. Despite increase of glycerol accumulation as by-product of biodiesel production, it is unprofitable to purify this polyol for subsequent application in food and cosmetic industry. There are known *Candida* yeast strains effectively converting glucose to glycerol, however, they need aeration which elevates costs of process approximately twice. Therefore there is an interest in development of microbial or yeast strains effectively converting cheap feedstocks to glycerol under anaerobic conditions. Facultative anaerobic yeast *Saccharomyces cerevisiae* can be a good platform for development of such recombinant strains. In *S. cerevisiae* glycerol synthesis occurs from dihydroxyacetone phosphate by subsequent action of glycerol-3-phosphate dehydrogenase (*Gpd1*) and glycerol-3-phosphate phosphatase (*Gpp2*). Dihydroxyacetone phosphate predominantly isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase (*Tpi1*) and subsequently converted to ethanol. To redirect consumed carbon toward glycerol instead of ethanol we aimed to construct recombinant strains with simultaneous decrease of *Tpi1* and increase of *Gpd1* and *Gpp2* specific activities. To decrease *TPII* gene expression recombinant *S. cerevisiae* strains with shortened versions of *TPII* gene promoter to 100, 50 or 25 base pairs were constructed. Constructed strains revealed sequential decreases in *Tpi1* activity. Strains with 50 or 25 bp version of *TPII* promoter possessed up to 2 times increase in glycerol production in comparison with WT strain. In order to enhance the activities of enzymes involved in glycerol synthesis, we transformed *S. cerevisiae* with vectors containing gene *GPD1*, or *GPP2*, or hybrid *GPD1-GPP2* ORF (encoding artificial fusion of both enzymes) under the control of strong constitutive promoter of the alcohol dehydrogenase gene (*ADH1*). Recombinant strains overexpressing *GPP2* gene didn't reveal essentially higher glycerol production than WT strain. Recombinant strains overexpressing *GPD1* or *GPD1-GPP2* fusion genes showed diverse increase in glycerol production. Glycerol production reached 4 folds increase in the best of studied strain expressing *GPD1-GPP2* fusion. Combination of *GPD1-GPP2* fusion overexpression with *TPII* promoter partial substitution resulted to 5-fold increase of glycerol production as compared to the WT strain.

PS6-53: Optimization of lipid accumulation in oleaginous yeasts using pure and crude glycerol

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Biodiesel is usually produced from food-grade plant oils by transesterification; however this production is not economically feasible since the final product results more expensive than the petro-diesel fuel. Microbial lipids can represent a valuable alternative feedstock for biodiesel production in the context of a viable bio-based economy. It has been well established that fatty substances are produced by a number of microorganisms, notably by certain yeasts and fungi. Oil yeasts have been described to be able to accumulate lipid up to 20% of their cellular dry weight and, among these, few have been reported to accumulate oil up to 80%. Oleaginous yeasts can accumulate intracellular lipids during cultivation on various agro-industrial wastes as crude-glycerol,

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a 10% (w/w) byproduct produced in the transesterification process of oils converted to biodiesel. It is reported in literature that different oleaginous yeast strains present different metabolic responses depending on the origin of the crude glycerol employed, which may result in inhibitory effect on the yeast cells growth. The present work studied crude glycerol (vs. pure glycerol) as carbon source for lipid production exploiting three different oleaginous yeasts: *Rhodosporidium toruloides* (DSM 4444), *Lipomyces starkeyi* (DSM 70295) and *Cryptococcus curvatus* (DSM 70022). The main objective was to develop a successful fermentative strategy for achieving a high lipid productivity avoiding or at least reducing the inhibitory effects due to crude glycerol. The additional objective was to use and compare different techniques, in addition to gas chromatography, for monitoring lipid accumulation over time. In particular, fluorescent microscopy, flow cytometry and FTIR microspectroscopy analysis were performed. All these are relatively quick approaches that do not require lipid extraction and that can individually provide specific information about the process of production. Here we show the results obtained with all these techniques and discuss how they can be very helpful both for the initial screening phase as well as for monitoring the effective production.

PS6-54: Exploitation of a evolution strategy to select yeast strains improved in glutathione production

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Yeasts have been largely explored as cell factories to produce substances for food and industrial biotechnological applications. Among these chemicals, glutathione (GSH) is an important antioxidant molecule involved in several processes, including the control of redox potential, protection against oxidative stress, detoxification and transport of organic sulfur. Due to its functional roles, GSH is widely used in the pharmaceutical, food and cosmetic industries. Recently, GSH has received growing attention also in the winemaking field, to control oxidative spoilage damage; to limit the amount of browning pigments; to avoid the formation atypical aging characters; and to exert a protective effect on various aromatic compounds. At present GSH is successfully produced on an industrial scale through fermentation by high GSH-producing *Saccharomyces cerevisiae* strains, and several methodological tools have been reported for increasing efficiency and yield of the bioprocess. In this study, we have applied an evolution-based strategy that combines the sexual recombination of spores with the application of molybdate Mo(VI), a sulfate analogue toxic for the cells at high concentration, as specific selective pressure, to generate evolved *S. cerevisiae* strains with enhanced GSH production. To achieve this aim we used the 21T2 wine strain from the Unimore Microbial Culture Collection (UMCC) and we exploited its resistance to Mo(VI) as a rapid and high-throughput screening method for the selection of the evolved strains improved in GSH production. By this strategy, we obtained two evolved strains, Mo21T2-5 and Mo21T2-12, both able to enhance GSH content in wine with an increase of 100% and 36%, respectively, compared with the parental strain 21T2, and 120% and 50% compared with initial GSH content in the must. Our strategy, unlike the standard evolutionary approaches, has the advantage of not requiring multiple rounds of screening and extensive cultivation periods because the evolved strains are recognized through a selectable phenotype. The Mo(VI) resistance has proved to be effective for the selection of the desired evolved strains, probably by activating the yeast common metal response that involves sulfur assimilation and GSH biosynthesis.

PS6-55: Comparative genomic analysis and phenotypic characterization of industrial *Saccharomyces cerevisiae* strains used in sugarcane-based fermentation processes in Brazil

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In Brazil, sucrose-rich broths (cane juice and/or molasses) are used to produce billions of liters of both fuel ethanol and *cachaça* per year, using selected *S. cerevisiae* industrial strains. We have studied the genetic characteristics of a group of 9 fuel ethanol and 5 *cachaça* industrial yeast strains that tend to dominate the

fermentors during the entire production season (allowing efficient and stable fermentations) by array comparative genomic hybridization. Widespread presence of *SUC* genes encoding invertase at multiple telomeres has been shown to be a common feature of both baker's and distillers' yeast strains, and is postulated to be an adaptation to sucrose-rich broths [1]. Our results show that only 2 strains (one fuel ethanol and one cachaça yeast) have amplification of the *SUC* genes, which allowed high invertase activity during growth on sucrose. The other industrial yeast strains had a single *SUC* gene (*SUC2*) in their genome, although they showed different patterns of invertase activity especially during growth under non-repressing conditions (glycerol/ethanol, and with low levels of glucose). These results indicate that invertase activity probably does not limit sucrose fermentation during fuel ethanol and *cachaça* production by these industrial yeast strains. Most of the telomeric *HXT* genes, which encode hexose transporters (*HXT8*, *HXT9*, *HXT11*, *HXT12*, *HXT15* and *HXT16*), were missing in the genome of the industrial strains, and many strains also had lower gene copy number of the high-affinity *HXT7* and *GAL2* encoded permeases. The telomeric *HXT* genes have been probably replaced by the amplified telomeric *SNO/SNZ* genes involved in pyridoxine and thiamin biosynthesis [2]. Indeed, all the fuel ethanol strains had amplification of these genes, while some *cachaça* yeasts that did not show amplifications of *SNO/SNZ* genes showed amplification of thiamin transporters (*THI7*, *NRT1* and *THI72* genes), highlighting the importance of this vitamin for efficient fermentation of sucrose-rich broths. Our data suggest that these gene amplifications provide an important adaptive advantage under the industrial sugar-rich fermentation conditions in which the yeast are used. Financial Support: CAPES, CNPq, FAPESP and NSF.

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PS6-56: Molecular toolbox for efficient engineering of industrial yeast cell factories

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Yeast *Saccharomyces cerevisiae* is one of the most promising hosts for Biorefinery applications. Biorefineries serve for the sustainable production of a wide range of fuels, chemicals and energy from various biomass feedstocks. In industrial scale, yeast strains with high fermentation capacity and increased tolerance against stress conditions encountered within the harsh industrial environment must be used. The development of genetically modified industrial strains suitable for converting the carbon from non-food waste streams into added-value products in third generation Biorefineries is an important challenge. However, a spectrum of genetic engineering tools suitable for industrial strains, although very broad when laboratory strains with well-defined genetic properties are engineered, is still limited due to their considerable genetic complexity. In this work, we present the development and application of a CRISPR-Cas9 approach for genome editing of strains with industrial background. The CRISPR-Cas9 method mediates a high efficiency modification of any sequence of choice resulting in marker-free one-step gene disruptions and, potentially, simultaneous insertions of heterologous gene(s) in unrelated industrial strains. Furthermore, we show the construction of second generation of integrative vectors enabling the delivery, stable integration and controlled expression of heterologous genes in strains isolated from various industrial settings. The applicability of the developed molecular toolkit for metabolic engineering of industrial cell factories by construction of lactic acid producing and C5-source utilizing industrial yeast strains will be demonstrated. This project is part of BioREFINE-2G (www.biorefine2g.eu), which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613771).

PS6-57: *NFS1* is involved in control of *Saccharomyces cerevisiae* resistance to isobutanol

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Concerns about energy security and climate change have incited interest in production of biofuels from renewable resources. Isobutanol has received great attention as a potential biofuel because it can be used both pure and mixed with gasoline, has higher energy density, lower hygroscopicity, higher octane value and is less volatile relative to ethanol. Yeast *Saccharomyces cerevisiae* is a promising organism for isobutanol production; however, it is rather susceptible toward high isobutanol concentrations. Molecular mechanisms conferring isobutanol resistance and sensitivity have not been elucidated yet. We believe that identifying specific genes

involved in these pathways with subsequent construction of more resistant yeast strains could serve as a useful strategy to increase isobutanol yield. To address this question in an unbiased manner, we used insertional mutagenesis and searched for mutant clones with altered sensitivity to isobutanol, i.e. either with elevated or decreased tolerance to isobutanol relative to the wild-type strains. Among others, a resistant strain 95 was isolated able to grow in the medium containing 4% isobutanol. To identify the disrupted genomic locus, we digested the chromosomal DNA of strain 95 with rare-cutting XbaI enzyme, self-ligated the fragments and used them for *E. coli* transformation. The insertion plasmid along with the flanking regions was isolated from antibiotic-resistant bacterial clones. Sequencing of the flanking regions showed that the insertion cassette damaged *NFS1* gene separating 16 amino acids from the C-terminus of Nfs1 protein. *NFS1* encodes a cysteine desulfurase involved in the biogenesis of iron-sulfur (Fe/S) cluster proteins and known to play a direct role in thio-modification of mitochondrial and cytoplasmic tRNAs. So far there has been no evidence linking these processes to alcohol tolerance in yeast. It is essential to confirm that the observed isobutanol resistance of strain 95 is a result of the insertion cassette integration, rather than a secondary mutation elsewhere in the genome. To prove this, we attempted to rescue the phenotype by transforming strain 95 with a plasmid harbouring the wild type allele of *NFS1* gene. In addition, the deletion cassette for *NFS1* gene was constructed and introduced into the wild type strain BY4742. The characterisation of the obtained strains is now in progress.

PS6-58: Construction of stable recombinant industrial yeast strains that secretes glucoamylase and alpha-amylase

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Some years ago in our laboratory, a yeast genetic transformation system was developed that allows the introduction of multiple copies of a desired gene expression cassette into the genome of laboratory or industrial *S. cerevisiae* strain. The transformation occurs by delta-integration promoted by a DNA fragment containing the desired gene expression cassette flanked by δ-sequences lacking any positive selection marker. Employing co-transformation with the pAJ50 plasmid (could be other) it was possible the selection by auxotrophic complementation of *leu2* mutation and/or Geneticin resistance (G418R) transformants [1]. Initially, the vector containing the glucoamylase gene of *Aspergillus awamori* (δ Gluco δ) was used to transform haploid and diploid yeast strains of laboratory. Then, this vector was also used to transform PE-2, one of the yeast strains most widely used for industrial ethanol production in Brazil [2]. It were obtained PE-2 recombinant clones harbouring 1-16 copies of the inserted cassette showing 100% stability after 80 generations [1]. More recently, the *Bacillus subtilis* α-amylase gene was inserted by δ-integration into the genome of *S. cerevisiae* strains of laboratory. To achieve this, we constructed two transformation vectors containing: 1) the truncated α-amylase gene of *B. subtilis* (amyEt) with its own signal sequence, under the regulation of the *ADH1* promoter and terminator of *S. cerevisiae* (δ AmyEt δ); 2) the complete α-amylase gene of *B. subtilis* (amyE), with the signal sequence of MFα of *S. cerevisiae* under the regulation of *PGK* promoter and terminator (δ AmyE δ). These vectors were used for genetic transformation of *S. cerevisiae* strains, in co-transformation with pAJ50 plasmid that allows positive selection. The transformant clones grown on solid medium YPDA (0.5% starch) produced amilolise halos of different sizes. These results indicate that these vectors have great potential to be used in the genetic transformation of industrial wild-type strains of *S. cerevisiae* as PE-2 and others. We are now building derived recombinant clones of PE-2 strain containing multiple copies of both δ Gluco δ and δ AmyE δ and, on laboratory scale, we are looking for the identification and selection of the better ones that are able to ferment starch.

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[1] Guerra, O.G. et al.(2006) *J. Microbiol. Methods.* 67, 437-45, 2006; [2] Basso L.C. et al. (2008) *FEMS Yeast Res.*, 8,1155-63, 2008.

PS6-59: Comparative proteomic and DNA microarray analysis of Lager brewer's yeast in the process of autolysis**Jinjing Wang, Qi Li, Weinan Xu***Jiangnan University, China*

The autolysis of lager brewer's yeast during beer production significantly affects the quality of final product. In this work, we performed proteomic and microarray studies on lager brewer's yeast to examine changes in translation and transcription levels in the process of autolysis. Protein and RNA samples of the strain Qing2 at two different autolysis stages were obtained for further study. Ultimately 49 kinds of proteins were considered to be involved in autolysis-response, among which 8 were up-regulated and 41 were down-regulated. Results of comparative proteome analysis showed that important changes had taken place as an adaptive response to autolysis. Functional analysis showed that the carbohydrate and energy metabolism, cellular amino acid metabolic processes, cell response to various stresses (such as oxidative stress, salt stress, and osmotic stress), translation and transcription were repressed by the down-regulation of correlative proteins, whereas starvation and DNA damage responses might be induced. The comparison of transcriptome and proteome data demonstrated that most autolysis-response proteins had general coordination between transcription and expression levels. Thus these proteins were thought to be transcriptionally regulated. These findings provide important information about how lager yeast acted to cope with autolysis at molecular levels, which might enrich the global understanding of autolysis process.

PS6-60: Modification of γ glutamylcysteine synthetase as a tool for construction of glutathione overproducers in yeast *Hansenula polymorpha***Marianna Yurkiv¹, Olena Kurylenko¹, Roksolana Vasylyshyn¹, Kostyantyn Dmytruk¹, Andriy A. Sibirny^{1,2}**

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Glutathione (γ -L-glutamyl-L-cysteinyl-glycine; GSH) is a tripeptide with different physiological functions in eukaryotic cells. Most of these functions have been related to its antioxidative properties caused by the thiol group in the cysteine moiety. Due to the antioxidative properties of GSH there is an increasing interest for application of this tripeptide in several industrial areas, including cosmetics, pharmaceutical products and foods. As an active ingredient of food, drugs and cosmetic products, GSH could alleviate harmful oxidative processes, scavenge toxic compounds at different kinds of human intoxications and strengthen whitening, skin repair antiaging effect. Microbial production of GSH using genetically engineered yeast strains and precursor amino acid supplementation has potential to satisfy the increasing industrial demand of this tripeptide. Microbial GSH overproduction is limited by mechanisms of feedback inhibition of γ -glutamylcysteine synthetase (GCS), the first and rate-limiting enzyme of GSH biosynthesis, by the end product. In addition the expression of gene coding for GCS is repressed by GSH. The methylotrophic yeast *Hansenula polymorpha* is regarded as a rich source of GSH due to the role of this thiol in detoxifications of key intermediates of methanol metabolism. In this work the selection scheme providing generation of GCS insensitive to feedback inhibition was developed. The modified versions of *GSH2* gene obtained by error prone PCR were cloned under the control of strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase in replicative plasmid pYT3. Selected transformants were analyzed for their resistance to different prooxidant agents (1,2,3-triazole, diethylmaleate, ethionine) as compared to strains carrying unmodified *GSH2* gene. Strains providing more intensive growth on the selective medium revealed higher GSH accumulation as compared to strains carrying unmodified *GSH2* gene, indicating the reduction of Gsh2 feedback inhibition. Sequencing of the one mutant *GSH2* allele enabled to identify five amino acid substitutions in the highly conserved Gsh2 domain. Detection of *GSH2* gene mutations leading to the elimination of negative regulatory mechanisms of GSH biosynthesis will create a competitive producer of this tripeptide.

Poster Session 7: Yeast as a model for human disease and drug testing

PS7-1: Aspirin and Salicylate effects on yeast cell proliferation

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Several nonsteroidal anti-inflammatory drugs (NSAIDs) also exhibit significant antineoplastic behaviour in mammalian cancer cells. Aspirin (acetylsalicylic acid) is widely investigated as pharmacological agent for the chemoprevention of colorectal cancer and other malignancies, with numerous clinical trials being already active. The mechanisms which mediate the anti-neoplastic effects of NSAIDs are only partially known. Importantly, NSAIDs can induce tumour cell death via pathways that are independent of Cyclooxygenase. The elucidation of these mechanisms has involved the use of a broad range of experimental models, including *Saccharomyces cerevisiae* cells. Indeed, yeast cells have been successfully used to study some of the toxic, growth inhibitory, proapoptotic effects of aspirin and diclofenac [2-(2,6-dichloranilino) phenylacetic acid] (reviewed in Farrugia G & Balzan R. [1], and van Leeuwen JS et al. [2]). We are investigating the in vivo effect of aspirin and its main metabolite salicylic acid on yeast proliferation. In our experimental conditions the exponential phase of cell growth appears to be largely unaffected when yeast is treated with salicylic acid. In contrast, the timing of G0 exit (lag phase) as well as key cell features characterizing the entry into G0 (such as cell density of cultures, budding index, cell size and cell viability) are altered by the drug. We are presently focused on getting a detailed picture of the above phenotypes and investigating the interaction of salicylic acid with known yeast signal pathways. In appropriate conditions, we are also comparing the salicylate effects with those induced by aspirin.

[1] Farrugia G & Balzan R. (2013) *Oxid Med Cell Longev*. Art. ID 504230; [2] van Leeuwen JS et al. (2012) *Curr Drug Metab* 13, 1464-1475.

PS7-2: Yeast model for drug discovery: Identification of molecules acting as potential therapeutics for POLG-related diseases

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Mutations in POLG, encoding mitochondrial DNA polymerase, are a major cause of mitochondrial disorders including the lethal Alpers' syndrome, progressive external ophthalmoplegia, sensory neuropathy, ataxia and parkinsonism. To date, no effective therapy is available. Based on the conservation of mitochondrial function from yeast to human, we used *Saccharomyces cerevisiae* harboring mutations in MIP1, the yeast POLG orthologous gene, as a tool to identify chemicals, which suppress mtDNA instability due to these mutations corresponding to human pathological substitutions. For this test, a thermosensitive mutant, mip1G651S strain, which is unable to grow on non-fermentable carbon sources at 37°C, was tested against a FDA approved chemical library of 1500 molecules to find drugs able to restore the growth. Six molecules, called MRS1-6, were found as able to rescue the thermosensitive phenotype of mip1G651S allele. The effects of one of them, MRS3, were further studied. We found that MRS3:

- 1) strongly reduces the petite frequency due to all the mutations inserted in MIP1, independent from the domain in which the mutations localize;
- 2) is not mutagenic for mtDNA;
- 3) does not rescue other mutants that affect the mtDNA stability unrelated to mtDNA replication;
- 4) rescues mip1-induced mtDNA instability through a mechanism distinct from the dNTP pool availability;
- 5) strongly increases the respiration rates either in wt and in mutant mip1 strains;
- 6) stabilizes Mip1 protein, increasing its levels;
- 7) increases the number of replicating-mtDNA.

Based on these results, further experiments have been then performed in other models: in *Caenorhabditis elegans*, in which MRS3 protects the worm from the physiological effects due to polg-1 deletion, and in fibroblasts from affected patients, where an increase of the mtDNA levels after treatment with MRS3 was observed. From these results, MRS3 can be considered as a promising drug for treating POLG disorders.

EB and LP contributed equally to this work, TL and AD contributed equally to this work.

PS7-3: Carbon source metabolism modulates effects of calcium depletion on cell fate

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Besides being an allosteric cofactor required for many enzymatic reactions, calcium plays a relevant role as a second messenger regulating a wide variety of cellular processes in virtually all eukaryotic organisms. Maintenance of intracellular Ca²⁺ homeostasis and a precise regulation of calcium-triggered signaling mechanisms are therefore crucial to the survival of all organisms. In this study we analyzed the effects of the ion shortage on various aspects of yeast physiology, including cell cycle progression, cell morphology, stress resistance and the proteomic and metabolomics profile. In *Saccharomyces cerevisiae* calcium depletion leads to slow growth, altered cell cycle progression, reduced cell size, abnormal vacuolar morphology and generates a state of oxidative stress likely resulting from accumulation of unfolded proteins within the lumen of the endoplasmic reticulum (ER stress, which in yeast has been shown to be associated with ROS production and cell death) that decreases cell viability and shortens chronological lifespan. The physiological effects of calcium shortage are strictly connected to the metabolic state of the cell: in fact, they are most evident during growth on rapidly fermentable sugars and can be mitigated by limiting the glycolytic flux rate (e.g. by growth in low glucose medium (calorie restriction), by mutations in the sugar uptake system or by inactivation of the hexokinase- or pyruvate kinase encoding genes. The overall glycolytic flux appears to be strongly reduced in cells cultivated in 2% glucose under calcium shortage, possibly as a result of oxidative damage of the glycolitic enzymes: under this condition, the reduced energetic efficiency of the glycolytic metabolism (lower ATP yield) combined with the reduced production of many “building blocks” (amino acids) synthesized from glycolytic intermediates may be insufficient to sustain the fast growth rate typical of fermenting yeast cells. Molecular and physiological analyses of the effects of calcium depletion highlight a conflict between a reduction in glycolytic flux (in the absence of a switch to respiratory metabolism) and sustained anabolic reactions (notably protein synthesis) that originates ER stress, leading to ROS production and cell death. The above described results that make use of biochemical, post-genomic and genetic investigations, together with ongoing FBA modeling will allow system-level understanding of this important topic.

PS7-4: Identifying in yeast the counterpart of the mammalian EGFR

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The yeast *Saccharomyces cerevisiae* is a well-known model for higher eukaryotes molecular processes based on a high degree of conservation of many signalling pathways as compared to mammalian cells. Yeast Ras/cAMP/PKA pathway is one such case, controlling proliferation, life span and differentiation. *S. cerevisiae* has two Ras proteins, Ras1 and Ras2 with similar functions but differently regulated. Their N-terminals share considerable homology with the mammalian Ras proteins[1], which are able to substitute for Ras1 and Ras2 in the activation of adenylyl cyclase Cyr1[2]. In mammalian cells Ras/Raf/MEK/ERK pathway responds to extracellular signals, namely EGF growth hormone through the correspondent receptor EGFR, which dimerization pattern depends on the type of external ligand. In yeast, instead, the upstream effector of Ras proteins is unknown. To identify the yeast correlate of the mammalian EGFR, two approaches were used. (1) A detailed database search for EGFR conserved domain architecture using a cell surface subset of proteins. (2) The total proteome of *S. cerevisiae* wt, *ras1Δ* and *ras2Δ* mutants, was blotted against anti-EGFR antibody. Additionally, blotting was also done against Erbitux® (Merck), used in the treatment of colorectal cancer, which active ingredient is Cetuximab, a human/murine chimera antibody that targets/blocks EGFR. Five candidate proteins were identified: the heat shock proteins Ssa2 and Ssb2, and the glycolytic glyceraldehyde-3P-dehydrogenase3 (Tdh3) and pyruvate decarboxylase Pdc1/5. All these proteins share 3 of the 8 conserved aminoacids required for binding Cetuximab. Further research includes the use of mutants for repeating blotting and site-directed mutagenesis for identification of the residues that are responsible for binding of the anti-EGFR.

[1] Tamatoi F et al. (2011) Genes Cancer 2(3): 210-15; [2] Nielsen KL et al. (2001) Oncogene 20(17): 2091-100.

[§]PhD Student (ESR) of the Marie Curie Initial Training Network Glycopharm (PITN-GA-2012-317297).

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PS7-5: Yeast as a model system for diseases associated with defective coenzyme A metabolism

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Mutations in nuclear genes associated with defective Coenzyme A biosynthesis have been identified as responsible for some forms of neurodegeneration with brain iron accumulation (NBIA), namely PKAN and CoPAN. PKAN are defined by mutations in *PANK2*, encoding the pantothenate kinase 2 enzyme, that account for about 50% of cases of NBIA, whereas mutations in CoA synthase *COASY* have been recently reported as the second inborn error of CoA synthesis leading to CoPAN. To investigate if defective CoA metabolism could underlie a more general disequilibrium of lipid metabolism and mitochondrial dysfunctions and its relationship with brain iron accumulation, we have performed phenotypic and biochemical investigation in a recently developed yeast model expressing the pathogenic missense mutation *COASY*^{R499C} found in CoPAN patients. The results obtained showed that yeast mutant defective in CoA biosynthesis has altered mitochondrial function, lipid content and iron metabolism thus partially recapitulating the phenotypes found in patients and establishing yeast as a potential model to help elucidating the pathogenesis underlying this disease.

PS7-6: *S. cerevisiae* as a tool to select inhibitors of the deneddylation activity of the COP9 signalosome

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The COP9 signalosome (CSN) protein complex plays a key role in regulating cullin-RING ligases and is a central mediator of cellular functions essential for cancer progression. The *CSN5/Jab1* gene, encoding the catalytic subunit of the complex, has been found amplified in many tumors; however, due to its pleiotropic effects, it has been difficult to dissect Csn5 function and its involvement in cancer progression. Moreover, while a growing body of evidence point to the neddylation pathway as a good target for drug development, specific inhibitors have not yet been developed for the Csn5 enzyme. Deneddylation by the CSN is conserved in the budding yeast *Saccharomyces cerevisiae* and, in contrast to human or plants, lack of Csn5 does not compromise viability of budding yeast. We have recently performed a transcriptomic and proteomic analysis of a Δ csn5 strain to assess its function in budding yeast, and we have shown that Csn5 is involved in the modulation of the genes controlling amino acid and lipid metabolism and in particular of the ergosterol biosynthesis; this observation correlates with lower ergosterol level in Δ csn5 cells [1]. In the study shown here, we have used budding yeast as a model to identify novel inhibitors of Csn5 deneddylating activity. We present our preliminary results obtained using a combined approach of molecular modelling and simple genetic tools to identify small molecules as selective inhibitors of Csn5 deneddylating function.

[1] Licursi et al., (2014) *FEBS Journal*, 281, 175-190

PS7-7: *In vivo* selection of *JARID* histone demethylases inhibitors and their use to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation

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Histone demethylases have a prominent role in epigenetic regulation and are emerging as potential therapeutic cancer targets. In order to discover inhibitors specific for H3K4 histone demethylation we set up a screening system which tests the effects of candidate small molecules inhibitors on a *S.cerevisiae* mutant strain which requires Jhd2 demethylase activity to efficiently grow in the presence of rapamycin. In order to validate the system we screened a library of 45 structurally different compounds designed as competitive inhibitors of α -ketoglutarate (α -KG) cofactor of the enzyme, and found that one of them, compound RS3195, inhibited Jhd2 activity *in vitro* and *in vivo*. The same compound effectively inhibits human *JARID 1B* and *1D* *in vitro* and increases H3K4 tri-methylation in HeLa cells nuclear extracts. When added *in vivo* to HeLa cells, the compound leads to an increase of tri-methyl-H3K4 but does not significantly affect H3K9 and H3K27 tri-methylation. On the same cells we observed a strong cytostatic effect at 30 μ M, concentration at which around 47% of the cells remained blocked in G2/M. At the same concentration, compound RS3195 induced a mild cytotoxicity, and a moderate apoptogenic effect. Similar effects were observed in the MCF7 breast cancer cell line which over-expresses *JARID 1B* whose K4-demethylase activity appears related to cancer cells proliferation. In order to better understand the role of H3K4 methylation in transcription regulation we are currently testing the transcriptomic effects of RS3195 and other demethylase inhibitors in yeast and in mammalian cell lines. In conclusion, the inhibitor RS3195, differently from other known inhibitors which provoke a general increase of methylation at all H3 lysine residues, appears to be specific for H3K4 demethylation *in vivo*. A direct relationship between this inhibitory action and the observed cytostatic effect as well as RS3195 in depth mechanism of action still remain to be fully elucidated. Our selection system may provide a new robust tool for the discovery of effective H3K4-specific HDM inhibitors.

[1] Mannironi C, Proietto M et al. (2014) *PLoS One* 9, e86002.

PS7-8: New insights on trehalose metabolism: Trehalose-6-phosphate as a candidate for drug design

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Trehalose has been found in bacteria, fungi, plants, insects and invertebrates. This sugar stabilizes and protects membranes and proteins, increasing cell tolerance to adverse conditions. In pathogenic organisms, the simple ability to withstand severe environmental stresses is mandatory for their survival in humans. Since this pathway is entirely absent in mammalian cells and makes use of highly specific enzymes, trehalose metabolism might be an interesting target for antibiotics. The most usual pathway of trehalose synthesis involves two enzymes: trehalose-6-phosphate synthase (Tps1), which catalyzes the synthesis of trehalose-6-phosphate (T6P), and trehalose-phosphatase (Tps2), which dephosphorylates T6P to trehalose. The complex of synthesis in the yeast *Saccharomyces cerevisiae* also includes two other proteins, Ts11 and Tps3, which seem to have regulatory functions. In this work, the effect of T6P on trehalose synthesis was tested, using *S. cerevisiae* as model. The metabolism of trehalose in this yeast shows striking similarities with that of other organisms: several *S. cerevisiae* proteins have been shown to functionally replace orthologous proteins and vice versa. We observed that, in extracts of heat stressed cells, Tps1 was inhibited by T6P and by ATP. Mg²⁺ in the presence of cAMP. In contrast, cAMP-dependent phosphorylation did not inhibit Tps1 in tps3 cells, which accumulated a higher proportion of T6P after stress. Tps2 activity was not induced in a tps3 mutant. Taken together these results suggest that Tps3 is an activator of Tps2. However, to perform this task, Tps3 must be non-phosphorylated. This

mechanism would be important to readily stop trehalose synthesis during recovery from stress. With the end of the stress, Tps3 would be phosphorylated by cAMP-dependent protein kinase, decreasing Tps2 activity and, consequently, increasing the concentration of T6P which, in turn, would inhibit Tps1. According to our results, T6P is an uncompetitive inhibitor of *S. cerevisiae* Tps1, able to decrease the rate of reaction to zero at saturating concentrations. We also tested the effect of T6P on Tps1 of *C. albicans*. In the presence of 125 µM T6P, the induction of *C. albicans* Tps1 caused by a heat stress was reduced in 60%. Due to the similarities found in terms of sequence and function between Tps1 of *S. cerevisiae* and several pathogens, these results suggest that the use of T6P is very promising regarding the treatment of infectious diseases.

PS7-9: Homology curation at SGD: Yeast and yeast research inform genetic medicine

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The foundation for much of our understanding of basic cellular biology has been learned from the budding yeast *Saccharomyces cerevisiae*, and studies with yeast have provided powerful insights into human genetic diseases and the cellular pathways in which they are involved. This utility of yeast as a model for human disease arises from the biochemical unity that underlies all forms of life. Yeast has become extremely useful in the study of various diseases that afflict humans, such as cystic fibrosis, kidney disease, mitochondrial diseases, and neurodegenerative diseases such as Parkinson's. Recent work with humanized yeast (in which yeast genes have been replaced with human orthologs) and humanized yeast proteins (in which key residues have been altered to match the human sequence) has demonstrated extensive conservation of ancestral functions through time and across taxa. We will present an update on new developments at the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org), the premier community resource for budding yeast. In order to promote and support the ways in which yeast and yeast research can inform genetic medicine, we are providing comprehensive curation for human disease-related genes and their yeast orthologs, including high quality manually curated information regarding functional complementation and conserved function. We also associate sequence changes with variations in yeast phenotypes and corresponding human disease manifestations. Curated information for yeast genes will be displayed on new Homology pages at SGD. Curated information for human genes will be available from the new "Yeast to Human Portal" knowledge center at humanportal.org. This new information is provided in ways that allow data mining and encourage innovation, for researchers studying both yeast and other organisms. These expanded 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).

PS7-10: *Kluyveromyces lactis*: a good model to study the molecular basis of Hailey-Hailey disease

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Hailey–Hailey disease (HHD), also known as familial benign chronic pemphigus, is a rare, chronic and recurrent blistering disorder, histologically characterized by suprabasal acantholysis. The skin lesions usually appear around puberty and no later than the third to fourth decades of life. The genetics and pathophysiology of HHD have been linked to mutations in ATP2C1. The gene encodes for an adenosine triphosphate (ATP)-powered calcium channel pump. The encoded proteins belong to the SPCA (secretory pathway Ca²⁺/Mn²⁺-ATPase) subfamily of P-type ion motive ATPases. Calcium (Ca²⁺) is a ubiquitous intracellular signal responsible for controlling numerous cellular processes and the essential components of the cellular Ca²⁺ signaling machinery are conserved from yeast to human, including Ca²⁺ channels and transporters, Ca²⁺ sensors and signal transducers. In our work the genetically tractable *Kluyveromyces lactis* yeast, has been used to study the molecular basis of HHD disease. Yeast is a simplified model of eukaryotic cell so that it lacks redundancy of multiple isoforms and the complexity of splice variants that are characteristic of mammalian cells. Instead, there is a limited number of genes that may be deleted individually or in combination to decipher their exact role in

Ca^{2+} homeostasis and signaling. We previously reported that, similarly to human keratinocytes HHD-derived cells, in yeast the loss of *KIPMRI* promotes cellular toxicity caused by increased oxidative stress linked to the alteration of calcium homeostasis in the mutant cells. By a cDNA library derived from MDCK cells, a functional suppression screening of the *KIPMRI* deletion mutant was performed. We found that the Glutathione S-transferase \square -subunit (GST), an important detoxifying enzyme, could be a candidate gene associated with human Hailey-Hailey disease. Indeed, expression of GST in KlPmr1⁺ suppressed several yeast's mutant defects in addition to the oxidative-stress toxicity. Additionally, we have validated the discoveries made in yeast in HHD-derived keratinocytes cells. In fact, our analysis showed a decreased expression of the human GST counterpart (GSTT1) in HHD-lesional derived keratinocytes compared to non-lesional skin derived from the same patients. Moreover, we found that the frequencies of GSTT1-null genotype was increased in the HHD patients compared with healthy individuals ones.

PS7-11: Study of effectors proteins of *Campylobacter jejuni* using yeast as model

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Saccharomyces cerevisiae as a eukaryote cell model offers many advantages, including simple culture conditions and several molecular tools currently implemented for the study of this yeast. On the other hand, *Campylobacter jejuni* is one of the most common pathogens associated to gastroenteritis. This microorganism displays a large array of virulence factors. Among others, the presence of effector proteins and export systems, through which these proteins are directly injected into the host cytoplasm, where, the effector proteins produces a series of changes, which promote the bacterial invasion and contribute to its prevalence by allowing it to evade immune system. In this work, we use of *S. cerevisiae* to identify effectors proteins of *C. jejuni* and analyze their mechanisms of action for the design of treatments for the pathologies caused by *C. jejuni*. For that, the genome sequence of the *C. jejuni* strain M1 (highly virulent) and RM1221 were subjected to Reciprocal Best Hit (RBH) which allows the identification of orthologs in the coding sequences of the strains analyzed. Using this bioinformatics tools, 6 possible effector proteins were identified from the virulent strain M1 (CJM1_148, 203, 206, 480, 1321 and 1637). This proteins beside to other four proteinas obtained from bibliographic references (CiaB, HtrA, TssD and TssI) were cloned and expressed in *S. cerevisiae* where their effect were evaluated in the viability of the yeast in standard or under stress condition such as sorbitol, salts, caffeine and nocodazole. The results show the expression of 4 of the proteins reduces the growth of *S. cerevisiae*, which indicates that this proteins interferes with the normal functions of the yeast. Null mutants involved in this process was evaluated.

PS7-12: Phenotypic profiling of antifungal agents using multiparametric yeast signatures

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The increased use of existing antifungal agents has resulted in the development of resistance to these compounds and correspondingly boosted the necessity for newer antifungal drugs. To gain further insight into the development of antifungal agents, the phenotypic profiles of currently available antifungal agents of three classes-ergosterol, cell wall and nucleic acid biosynthesis inhibitors-were investigated using yeast morphology as a chemogenomic signature. Amongst others, we analyzed echinocandins (echinocandin B and micafungin), an azole (fluconazole), an allylamine (terbinafine), a morpholine (amorolfine) and a fluorinated pyrimidine analog (5-fluorocytosine). The comparison of drug-induced morphological changes with the deletion of 4,718 non-essential genes confirmed the mode of action of the drugs. Besides we found some unexpected morphological similarities between not only the yeast treated with ergosterol affecting agents and V-ATPase mutants but also wild type treated with cell wall acting drugs and *vma* mutants. To this effect, treatment of the drugs that interfere with the synthesis of ergosterol exhibited the reduction of vacuolar quinacrine fluorescence in the wild-type yeast cells, demonstrating the critical requirement of ergosterol for the V-ATPase activity. Incubation of yeast

cells with concanamycin A, a potent inhibitor of V-ATPase, and cell wall-affecting drugs prior to zymolyase treatment resulted in an increased sensitivity to an enzymatic cell wall degradation, suggesting the role of V-ATPase in fungal physiology is wide-ranging to influence the cell wall integrity possibly via its effect on the secretory vesicles haulage to the fungal cell tip. To improve, simplify and accelerate drug development, we developed a systematic classifier that sorts a newly discovered compound into a class with a similar mode of action without any mutant information. Using well-characterized agents as target unknown compounds, a high-content image-based profiling method successfully categorized these bioactive small molecules into their particular classes. As such, this phenotypic profiling method exquisitely complements the previous phenomic and chemogenomic methods. Based on our data, we suggest that morphological profiling can be used to develop novel antifungal drugs.

PS7-13: Pathological role of mutations in human MPV17: *Saccharomyces cerevisiae* as a model system

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MPV17 is an intriguing gene necessary for mitochondrial DNA (mtDNA) maintenance in human, mutation of which leads to a peculiar form of hepatocerebral mtDNA depletion syndrome (MDS), a genetic disorder characterized by a severe, tissue-specific decrease of mtDNA copy number. In spite that *Mpv17* mutations are prominent cause of MDS in humans, its function remains a baffling and challenging issue. Originally considered as a peroxisomal membrane protein, it was later demonstrated that *Mpv17* is an integral protein localized to the inner mitochondrial membrane, as also previously demonstrated for the yeast orthologue *Sym1*, identified as a heat shock protein with a role in ethanol metabolism and/or tolerance. Previous studies have shed some light on the role of *Sym1*: it was shown that it has an essential role in OXPHOS competence, glycogen storage, mitochondrial morphology and mtDNA stability in stressing conditions such as high temperature and ethanol-dependent growth, nevertheless the specific function remain elusive. Recent studies have shown, by blue-native PAGE, that *Sym1* takes part in a high molecular weight complex by interaction with partner proteins whose identity is presently unknown. In order to define the molecular basis and clarify the pathological role of *MPV17* variants associated with the human disorder, we have taken advantage of *S. cerevisiae* as a model system. We studied the effect of seven alleged pathological mutations that are conserved between the two proteins on the cell physiology, in particular on mitochondrial function, showing that all the *Sym1* recombinant variants lead to an OXPHOS defect and to a drastic increase of mtDNA instability. These results allow to validate the mutations as the cause of the disease. The data obtained are in agreement with the patients molecular phenotype. Furthermore we analyzed the molecular effects of the mutations on the protein determining (i) the stability of the protein (ii) the capability to be imported to the mitochondria and (iii) the ability to take part to the high molecular weight complex. Our results indicate that none of the mutations prevent the correct mitochondrial localization of the protein. In contrast some mutations cause either the protein instability or prevent the entrance in the complex or both. Overall, the results obtained give informations on the molecular mechanisms by which the different mutations lead to the disease.

PS7-14: Investigation of molecular mechanisms of action of Valproic acid, an anticancer drug using budding yeast as a model organism

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Valproic acid (VPA) is a broad-spectrum histone deacetylase (HDAC) inhibitor, widely used for the treatment of bipolar disorders and epilepsy. Recently, HDAC inhibitors emerged as the most promising therapeutics for cancer. Besides the clinical assessment, the anticancer activity of VPA has been reported on multiple cancer cells *in vitro*. Although VPA was known to exert various biochemical effects, its mode of action remains elusive. Hence, this study aimed to unravel the comprehensive cellular processes affected by VPA and its molecular targets *in vivo* using budding yeast as a model organism. Interestingly, our genome-wide transcriptome analysis of yeast cells treated with a sub-lethal dose of VPA (6mM) showed differential regulation (1063 genes induced; 901 genes repressed) of 35% of the genome in approx. Functional enrichment analysis of VPA induced

transcriptome showed gene clusters belonging to various cellular processes including cell cycle, cell wall biogenesis, development, DNA damage repair, ion homeostasis, metabolism, stress response, transcription regulation and transport, whereas VPA repressed transcriptome revealed ribosomal biogenesis, macromolecules biosynthesis and metabolism, translation regulation genes. Moreover, results of our genetic screening identified molecular targets of VPA belonging to oxidative and osmotic stress response, cell wall integrity, iron homeostasis and histone modifiers. We have also detailed the activation of Hog1 (p38), a mitogen-activated protein kinase upon VPA treatment. Significantly, VPA transcriptome also evidenced a novel activation of genes related to glucose limitation (including hexose transporter genes), which is a promising approach for developing anticancer drugs. Altogether, our transcriptome and genetic screening analysis revealed the novel molecular insights of VPA action including its probable therapeutic targets. Additionally, we have demonstrated the role of canonical histones in mediating VPA action by employing synthetic histone H3/H4 mutant library. Our results disclosed crucial H3/H4 point, truncated mutants that conferred severe sensitivity and resistance to VPA induced stress. This study reveals a novel mechanism of mediating multiple cellular effects of VPA through histone residues. In conclusion, this study strengthens the current knowledge, advanced our understanding of comprehensive mechanisms of action of VPA *in vivo* and mechanistic links to propose it as a potent anticancer agent.

PS7-15: Assessment of allyl alcohol (Acrolein) cytotoxicity using global analysis of transcriptome and synthetic histone H3/H4 library of *Saccharomyces cerevisiae*

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All cells adapt to the environmental challenges by modulating global gene expression, thus their functional output. Allyl alcohol (AA) is a synthetic intermediate regularly utilized in chemical industries, one of the toxic environmental pollutants used as an herbicide in agriculture. AA transforms *in vivo* to Acrolein (Acr) by enzymatic activation. Acr is a major component of cigarette smoke, highly reactive and found ubiquitously in the environment. The exposure to AA/Acr is fatal and has detrimental effects; the mechanisms of its cytotoxicity remain elusive. In this study, we aimed to delineate the AA/Acr cytotoxicity mechanisms *in vivo* using budding yeast as a model organism. Global transcriptome profiling of yeast cells treated with a sublethal dose of AA (0.4 mM) showed differential regulation of 2213 genes (1171 induced; 1042 repressed). Functional classification of AA induced transcriptome revealed the enrichment of gene clusters belonging to developmental process, cell cycle, ion homeostasis, DNA damage repair, stress response, transport, cell wall biogenesis and metabolism, whereas AA repressed transcriptome showed enrichment of lipid metabolism, ribosome biogenesis, RNA processing and translation, macromolecules metabolism genes. Additionally, our results of genetic screening with AA/Acr identified its novel molecular targets, which are belonging to DNA damage repair, oxidative stress, cell wall integrity and iron homeostasis. Our results also detailed the role of mitogen-activated protein kinases (Hog1/p38, Slt2/p44) activation, functional cell wall integrity (CWI) pathway, and Mec1/Rad53/Dun1-checkpoint kinase pathway of DNA damage repair for tolerance against AA/Acr induced cytotoxicity. Interestingly, this study explored the use of ethanol and pyrazole (alcohol dehydrogenase inhibitor) as apparent antidotes for AA poisoning. Also, AA/Acr has demonstrated the reproductive toxicity by inhibiting yeast gametogenesis (meiosis). Moreover, we have employed the synthetic histone H3/H4 mutant library of yeast to understand the role of histone residues in mediating AA/Acr toxicity. Interestingly, the sensitivity of histone H3/H4 library mutants to AA/Acr revealed a novel mechanism of regulation of AA toxicity through histone residues. Altogether, our transcriptome and genetic screening results decipher the molecular cytotoxicity mechanisms of AA/Acr, facilitates the prediction of biomarkers for its toxicity assessment and therapeutic approaches.

PS7-16: Systematic identification of human/yeast complementation pairs to create a platform for testing tumor-specific variants

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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, model organisms like the budding yeast, *Saccharomyces cerevisiae*, can be utilized to fill this gap and serve as a platform for testing human genetic variants. In this instance, human/yeast cross-species complementation refers to the ability of a human gene to complement its yeast ortholog and rescue a loss-of-function phenotype. Given that rescue-of-lethality is the most straight-forward 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 create yeast expression vectors. 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. To this end, we performed two parallel screens, a one-to-one complementation screen for yeast essential genes implicated in chromosome instability and a pool-to-pool screen that queried all possible yeast essential genes for rescue-of-lethality by all possible human orthologs. Our work identified 65 human cDNAs that can replace the deletion of yeast essential genes including a non-orthologous pair *yRFT1/hSEC61A1*. We further chose four human cDNAs (*hLIG1*, *hSSRP1*, *hPPP1CA* and *hPPP1CC*) whose yeast orthologs function in chromosome stability and assayed 35 tumor-specific missense mutations in yeast for growth defects and sensitivities to DNA-damaging agents. The results of this study are both a candidate list of human genes whose genetic variants can be characterized in yeast, and a candidate list of somatic mutations that might contribute to chromosome instability in the tumor environment.

PS7-17: Quantitative analysis of NF-κB transactivation specificity using a yeast-based functional assay

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The NF-κB transcription factor family plays a central role in innate immunity and inflammation processes and is frequently dysregulated in cancer. We developed an NF-κB functional assay in yeast to investigate the following issues: transactivation specificity of NFκB proteins acting as homodimers or heterodimers; correlation between transactivation capacity and in vitro DNA binding measurements; impact of co-expressed interacting proteins or of small molecule inhibitors on NF-κB-dependent transactivation. Full-length p65 and p50 cDNAs were cloned into centromeric expression vectors under inducible GAL1 promoter in order to vary their expression levels. Since p50 lacks a transactivation domain (TAD), a chimeric construct containing the TAD derived from p65 was also generated (p50TAD) to address its binding and transactivation potential. The p50TAD and p65 had distinct transactivation specificities towards seventeen different κB response elements (NF-κB) where single nucleotide changes could greatly impact transactivation. For four NF-κB, results in yeast were predictive of transactivation potential measured in the human MCF7 cell lines treated with the NF-κB activator TNFα. Transactivation results in yeast correlated only partially with in vitro measured DNA binding affinities, suggesting that features other than strength of interaction with naked DNA affect transactivation, although factors such as chromatin context are kept constant in our isogenic yeast assay. The small molecules BAY11-7082 and ethyl-pyruvate as well as expressed IκBα protein acted as NF-κB inhibitors in yeast, more strongly towards p65. Thus, the yeast-based system can recapitulate NF-κB features found in human cells, thereby providing opportunities to address various NFκB functions, interactions and chemical modulators.

PS7-18: Yeast-based screens for modulators of a multidrug resistance pathway**Mikhail Keniya**¹, Andre Goffeau², David Maass¹, Richard Cannon¹, Brian Monk¹¹The Sir John Walsh Research Institute, University of Otago, New Zealand; ²Institut des Sciences de la Vie, Université Catholique de Louvain, Belgium

Multidrug resistance in pathogenic fungi poses significant risk to human health and agriculture. Lowering of intracellular xenobiotic concentration by the pleiotropic drug resistance (PDR) efflux pathway gives fungi sufficient time to develop gain-of-function mutations that enable high-level constitutive resistance due to overexpression of drug targets or efflux pumps. The model yeast *Saccharomyces cerevisiae* tolerates xenobiotics that activate the transcription factor Pdr1p by binding to its Xenobiotic Binding Domain (XBD). Interaction of activated Pdr1p with the KIX domain of the Mediator Complex enhances transcription of PDR transporters such as ScPdr5p. In order to develop an antifungal adjuvant that blocks the xenobiotic sensing pathway, we have established a screening system that identifies small molecule antagonists of Pdr1p-dependent activation. A green or red fluorescent reporter construct (Ura3-eGFP or Ura3-mRFP) was introduced at the PDR5 locus of a *S. cerevisiae* strain that contains the wild-type PDR1 gene and is hypersensitive to xenobiotics due to deletion of the Pdr3p transcriptional regulator and 7 drug efflux pumps. An isogenic strain with PDR1 deleted served as a control. The antifungal drug fluconazole (FLC) at growth-inhibitory concentrations induced cellular fluorescence 3-5 fold. Putative Pdr1p hits reduced FLC-induced fluorescence without inhibiting cell growth. A primary screen of a representative library of 2540 small molecules (obtained from National Cancer Institute, NIH, USA) gave six hits that were confirmed in two secondary screens. One secondary screen used rifampicin to induce Pdr1p without causing growth inhibition. The other secondary screen used the Pdr1p-induced expression of an apoptotic BCL-1 (BAX) protein from the PDR5 locus to identify antagonists that rescued cell growth. The screening system identified, efficiently, compounds that can be expected to assist the design of antifungals that are not susceptible to drug efflux induced by the Pdr1p transcriptional regulator.

PS7-19: Yeast as a system for modeling mitochondrial disease mechanisms and therapies**Roza Kucharczyk**¹, Anna Kabala¹, Katarzyna Niedzwiecka¹, Alain Dautant², Jean-Paul di Rago²¹Department of Genetics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; ²Univ. Bordeaux-CNRS, IBGC, UMR 5095, 1 rue Camille Saint-Saëns, F-33000 Bordeaux, France

Mitochondria, besides the key role in bioenergetics, carry out a lot of functions essential for cell viability, thus impairment of any of them can result in a wide spectrum of severe abnormalities in humans known as mitochondrial diseases. The diagnosis is difficult due to multiplicity of clinical manifestation depending on involved function and affected tissues. Additionally it is complicated by heteroplasmy of mitochondrial DNA (mtDNA) in human cells. The yeast *S. cerevisiae* is the organism of choice to uncover cellular and molecular mechanisms underlying the mitochondrial diseases. The most important is the capability to use fermentable carbon substrates as energy source, resulting in ability to survive even when mtDNA has been completely depleted, what more site-direct mutagenesis of mtDNA is possible by biolistic transformation and the population of mutated mtDNA will be 100% homoplasmic. ATP synthase is multi-subunit enzyme located in inner mitochondrial membrane. The enzyme uses the energy provided by the proton electrochemical gradient as a force to drive ATP synthesis. Point mutations in *ATP6* gene were identified in patients suffering the neurological defects. The mitochondrialy encoded Atp6 subunit of ATP synthase is evolutionary conserved, therefore it is possible to create yeast models of human diseases bearing the particular pathogenic mutations for analysis of their consequences. Here we present the results of systematic investigation on cellular effects of 9 pathogenic mutations introduced to *ATP6* gene of *S. cerevisiae* leading in human to Neurogenic Ataxia and Retinitis Pigmentosa (NARP), Leigh syndrome (LS), Charcot-Marie-Tooth (CMT), NARP or Familial Bilateral Striatal Necrosis (FBSN) syndromes. Importantly, chemical screens of drugs using yeast have pointed to potential therapeutic targets. Through selection of intragenic revertants in respiratory deficient mutants of *ATP6* gene, the identification of amino acids important for the mechanism of proton transport was possible. Thus from study of the pathogenic mutations yeast has brought us to the fundamental mechanism of the enzyme function.

PS7-20: Intracellular localization and cell cycle effect of cancer-related BRCA1 missense variants in yeast

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Germline monoallelic mutations within the tumor suppressor gene BRCA1 are associated with breast and ovarian cancer, about one-third (565/1,631) are represented by missense mutations, also called “variants,” that results in single amino acid change. Their relationship to disease is difficult to predict since the functional impact is not easily predictable. In order to investigate into the mechanisms involved in BRCA1-driven tumorigenesis, we think that functional assays taking advantage of the versatile eukaryote yeast *S. cerevisiae* could give us more information about the relation between the BRCA1 biological activity and tumor suppression. We already know that the expression of human wild-type BRCA1, neutral or pathogenic variants in the budding yeast *S. cerevisiae* has differential effect on recombination frequency which is an indication of DNA damage (Caligo *et al.* Hum Mutat. 2009), but we do not know the basic mechanism underlying this effect and if this is related to the tumor suppression function of BRCA1. We have studied the differential intracellular localization of BRCA1 wt and several missense variants through fluorescence microscopy, after inducing DNA damage by exposing yeast to methyl methanesulfonate (MMS). Then, have investigated the effect on the cell-cycle through FACS analysis of yeast strains expressing BRCA1 wild-type and cancer related variants. Preliminary results show that some cancer-related variants localize quite differently inside the cells as compared to BRCA1 wild type. MMS increased the nuclear localization of BRCA1 wt, while some cancer-related variants had a prevalent cytoplasmic localization; this could have an impact on BRCA1 functions such as on DNA-repair. FACS analysis showed that the expression of cancer-related variants of BRCA1 induced a cell-cycle arrest on G1/S phase. This may produce DNA damage as result of the stalling at the replication fork. In conclusion, although BRCA1 is absent in yeast, it may be implied in same pathway of DNA repair and cell-cycle control in yeast cells. This allow us to use this organism to better understand BRCA1 role and if we can make a relation to human pathogenicity.

PS7-21: Role of the oncogenic BRAFV600E in the osmostress response of yeast *Saccharomyces cerevisiae*

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Metastatic melanoma remains one of the most therapeutically challenging malignancies. A frequently mutated gene in melanomas is BRAF, carrying mainly the mutation V600E. BRAF is a kinase part of the RAS/RAF/MEK/ERK mitogen activated protein kinase (MAPK) signal transduction pathway. The V600E mutation determines a conformational change responsible for a constitutive activation of the protein. BRAFV600E-specific inhibitors have been shown to outperform conventional chemotherapeutic drugs. However, they are not immune of limitations, which need to be overcome by using drug cocktails. The aim of this study is to assess the functionality of the human BRAFV600E in yeast cells, in order to use yeast as a model system to identify novel BRAFV600E functional interactors that can be targeted for therapeutic purposes. Yeast does not have BRAF ortholog. Interestingly, yeast has the MEK counterpart PBS2 that encodes for a key player in the MAPK pathway responsible for the response to osmostress (Hog pathway). Therefore, we decided to assess the activity of wtBRAF, BRAFV600E and BRAFV600EΔ[3-10] (a splicing variant responsible for acquired resistance to BRAFV600E-specific inhibitors) in wild type (wt) yeast strains and in yeast strains deleted in genes encoding proteins involved in the Hog pathway. The three BRAF isoforms were cloned in the pYES2 plasmid under the control of the galactose inducible promoter. Yeast-expressed BRAFV600E has no overt effect on wt strain cell growth in standard medium, but it can confers a growth advantage when cells are grown on plates in which the NaCl concentration is increased to 1.8M. To further support the activity of BRAFV600E in the HOG pathway, we expressed BRAFV600E in a set of haploid BY4741 strains each one deleted in a specific gene involved in the HOG pathway. Interestingly, the expression of BRAFV600E in *hog1Δ* and *pbs2Δ* strains did not complement the strong growth defect in 1M NaCl. On the other hand, BRAFV600E expression in the double mutant *ste11Δssk1Δ*, allowed growth in the presence of 1M NaCl. Since Hog1, the target of Pbs2,

translocates into the nucleus after phosphorylation, we are constructing a *stellΔssk1Δ* mutant of the yeast strain expressing HOG-GFP to determine if BRAFV600E expression can rescue Hog1 translocation. The same experiments will be done also on cells expressing the other BRAF isoforms.

PS7-22: Evidence that the antiproliferative effects of Auranofin and other Organogold(III)-complexes in *Saccharomyces cerevisiae*

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Auranofin is a gold(I) based drug in clinical use since 1985 for the treatment of rheumatoid arthritis. Beyond its antinflammatory properties, Auranofin exhibits other attractive biological and pharmacological actions such as a potent in vitro cytotoxicity and relevant antimicrobial and antiparasitic effects that make it amenable for new therapeutic indications. For instance, Auranofin is currently tested as an anticancer agent in four independent clinical trials; yet, its mode of action is highly controversial. Other gold(III) compounds such as Auoxo6, AuL12, A₂phen and Aubipy_c are currently under study since they display antiproliferative and cytotoxic properties. With the present study, we explore the effects of these compounds in *S. cerevisiae* and their likely mechanism. We demonstrated that these compounds induced remarkable yeast growth inhibition, in particular in respiratory growth conditions. These data indicate that activation of mitochondrial metabolism greatly enhances yeast sensitivity to these metallo-drugs. In fact, a strong reduction of O₂ consumption is detected in yeast treated with these compounds. Concerning Auranofin the profound depression of cell respiration is indeed clearly documented as the main cause of cell death. Notably, the screening of selected deletion strains of genes, involved in mitochondrial function, allowed us to identify, several strains more resistant to AF treatment in comparison to the wild type strain. Among these, we identified the mitochondrial NADH kinase Pos5 as a primary target for Auranofin. We are currently evaluating if the other gold compounds share the same mechanism of Auranofin.

PS7-23: Use of a yeast-based functional assay to study P63, the long-lost cousin of the P53 tumor suppressor protein

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TP63 is a member of the TP53 gene family that encodes for different TA and ΔN isoforms (α, β, γ, δ and ε). P63 is a master regulator of gene expression for squamous epithelial proliferation, differentiation and maintenance: in fact TP63 germ-line mutations are responsible for a group of human ectodermal dysplasia syndromes (EDs). Moreover, P63 plays an active role in tumorigenesis. All P63 isoforms share an immunoglobulin-like folded DNA binding domain important for binding to sequence-specific response elements (REs), whose overall consensus sequence is similar to that of the canonical p53 RE. We took advantage of a yeast functional assay where a single P63 isoform can be expressed and its capacity to activate transcription from isogenic promoter-reporter constructs measured in order 1) to examine the contribution of RE sequence features to P63 isoform-dependent transactivation and 2) to functionally characterize TP63 alleles associated to Eds. We demonstrated that human wild-type TA- and ΔN-P63α proteins exhibited differences in transactivation specificity not observed with the corresponding P73 or P53 protein isoforms. These changes were dependent on specific features of the RE sequence and could be related to intrinsic differences in their oligomeric state and cooperative DNA binding. Furthermore, we highlighted the heterogeneity of P63 mutants in term of transactivation ability, interfering ability (i.e. the potential to inhibit the wild-type P63 when heterozygous) and temperature sensitivity on a subset of REs, underlining the importance of integrating clinical classification with functional parameters. All together, our results showed the high versatility of the yeast P63 functional assay in support of basic as well as clinical research.

PS7-24: Validation of a *MGM1/OPA1* chimeric gene for functional analysis in yeast of mutations associated with dominant optic atrophy

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Dominant optic atrophy (DOA) is a mitochondrial disease, characterized by mild to severe decrease in visual acuity, color vision deficiency and visual field defects, due to selective degeneration of retinal ganglion cells. DOA is a genetically inherited disorder associated, in most cases, with mutations in the *OPA1* gene encoding the mitochondrial GTPase of the dynamin family OPA1, a highly conserved protein primarily involved in mitochondrial fusion and in mtDNA maintenance. Opa1 is an integral protein of the inner mitochondrial membrane (IMM) and displays three highly conserved regions: a GTPase domain, a middle domain and a GTPase effector domain (GED). More than 200 pathogenic mutations have been identified so far which are spread throughout the entire *OPA1* gene. About 50% of these are missense mutations, mostly clustered in the GTPase domain, which cause heterozygous amino acid substitutions envisaged to exert a severe dominant negative effect. These latter mutations are often associated with a more severe syndromic disorder named “DOA-plus” which includes optic atrophy appearing in childhood, followed by chronic progressive external ophthalmoplegia (PEO), ataxia, sensorineural deafness, sensory-motor neuropathy, myopathy and mtDNA multiple deletions in adult life. In the yeast *Saccharomyces cerevisiae* *MGM1* (Mitochondrial Genome Maintenance) is the orthologous of *OPA1* gene. Mgm1 and OPA1 own equivalent functional domains, however, their amino acid sequences are poorly conserved. This is a limitation to the use of yeast for the analysis and validation of *OPA1* pathological mutations, since only few conserved substitutions found in patients can be introduced in the corresponding positions of the yeast orthologous gene. In order to find a model for the study of *OPA1* pathological mutations in *S. cerevisiae*, we produced a chimeric gene (CHIM3) encoding a protein composed by the N-terminal region of Mgm1 fused with the catalytic region of *OPA1*, in particular the whole GTPase region in which the majority of pathological mutations are localized. This *OPA1/MGM1* chimera was able to complement the oxidative growth defect of the *S. cerevisiae* *mgm1* deleted mutant, thus validating this construct as a model for the study of *OPA1* pathological mutations in *S. cerevisiae*.

PS7-25: Yeast-based drug discovery and development by the robot scientist “Eve”

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We have developed an automated yeast-based assay for high-throughput screens of antiparasitic targets. Briefly, we engineer yeast cells to be dependent on the expression of a heterologous enzyme that is either a parasite drug target or the human counterpart of that target. We then label up to 4 different strains with fluorescent proteins to allow the growth, in competition, of strains expressing 3 different parasite targets and their human ortholog. This pool is then treated with thousands of different compounds to allow the identification of hits, which inhibit the parasite target without affecting its human counterpart. These assays exclude cytotoxic agents and are readily automatable. We have exploited this yeast system in conjunction with a Robot Scientist called “Eve” that not only carries out the assays but also uses artificial intelligence to increase the efficiency of drug discovery. Using such assays, we have identified candidate drugs against specific targets in parasites that cause tropical diseases, such as malaria, African sleeping sickness, Chagas’ disease, Leishmaniasis, and elephantiasis. We will demonstrate the validation of these ‘hits’ using enzyme assays and parasites in culture and also show how well-known agents, such as the anti anti-cancer compound TNP-470, and the general antimicrobial triclosan, can be re-positioned for use against parasitic diseases as well as have their mechanisms of action clarified by these yeast-based assays.

PS7-26: Testing knockouts strains of *Saccharomyces cerevisiae* expressing ALS-linked VAPB^{P56S} for viability and proteostasis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons. P56S and T46I mutations in Vesicle-Associated membrane protein-associated Protein B/C (VAPB) are associated with Familial ALS type 8 (FALS8). The VAPB protein is located in the endoplasmic reticulum (ER) membrane. Despite not having a fully established function in mammals, its orthologous protein in *Saccharomyces cerevisiae*, SCS2, is involved in the ER morphology, phospholipids metabolism and unfolded protein response (UPR). Initially, we expressed human VAPB^{WT} and VAPB^{P56S} in *S. cerevisiae* under control of GAL1 promoter. Cells expressing VAPB^{P56S} displayed lower viability than the control strain in basal and stressful conditions. In order to test possible proteolytic degradation pathways of VAPB, we transformed different knockout strains with both VAPB^{WT} and VAPB^{P56S}: [^]ATG8, a deficient autophagy pathway strain; [^]PDR5, as PDR5 is involved in pleiotropic response to drugs as MG132, a proteasome inhibitor; and the conditional mutant of PRE1, which is unable to perform the correct proteasome assembly under treatment with doxycycline. Growth curves (OD_{600nm}) and serial dilutions were performed to assess the viability of these constructions. Again, lower viability was observed in strains expressing VAPB^{P56S}. This phenotype was more pronounced in knockout strains for the genes involved in the proteasome inhibition ([^]PDR5 + MG132 or PRE1 + doxycycline), when the final OD_{600nm} of mutant protein carrying strains reached at maximum 35% of the control. To assess a possible function of VAPB in the inositol metabolism, we expressed both VAPB^{WT} and VAPB^{P56S} in a [^]SCS2 strain, which presents inositol auxotrophy at temperatures above 34°C. The expression of VAPB^{WT} was able to suppress the inositol auxotrophy, whereas VAPB^{P56S} was not. As a parameter of redox state of the cells, the ratio of reduction to oxidized glutathione (GSH/GSSG) was measured by HPLC in normal conditions and under treatment with hydrogen peroxide. GSH/GSSG was two-fold in the control strain than in the VAPB^{P56S} strain in both cases. Finally, to assess UPR, the non-conventional splicing of HAC1 mRNA was measured by RT-PCR in BY4741 strains expressing VAPB^{WT} and VAPB^{P56S}. VAPB^{P56S} expressing cells showed higher quantities of the spliced form compared to VAPB^{WT} expressing cells and control strain, suggesting induction of UPR. Taken all together, these results indicate a general toxicity of VAPB^{P56S}.

PS7-27: Yeast and microgravity

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Exposure to a spaceflight environment can induce multiple changes in living systems, such as increased stress hormone levels, insulin resistance, altered immune responses, and abnormal musculoskeletal system structure and function, which can have undesirable effects on normal physiological processes. Microorganisms such as yeasts, because of their well-characterized genomes, robust viability, and ease of handling, are ideal models organisms for studying the effects of spaceflight conditions on eukaryotic cells. Understanding these effects allows to elucidate the processes underlying spaceflight related diseases and to develop suitable countermeasures to prevent them in long duration space missions. Many of these effects are similar to the alterations implicated in diseases of aging, but they occur and develop much more rapidly in space. The increase of knowledge of these processes could also improve life quality on Earth. In addition, microorganisms allow the study of the effect of microgravity on the reproduction and the creation of new species, thus stimulating the interest of evolutionary biologists. We participated in the 24-day FOTON-M3 space mission (2007) with the experiment SCORE (*Saccharomyces cerevisiae* Oxidative Stress Response Evaluation) to investigate the oxidative stress response of *S. cerevisiae* under conditions of real microgravity. We also participated in the 16-day STS-134 mission (2011)

with the experiment BioS-SPORE to investigate yeast sporulation and germination as well as the role of gravity in interspecific hybridization generation of new species. The results of both mission are presented.

PS7-28: Yeast-BRET: a high-throughput screening platform for the characterization of protein-protein interaction inhibitors

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Protein–protein interactions (PPI) are key players in the most crucial biological processes, both in physiology and pathology, and represent one of the major classes of “novel” therapeutic targets. PPI inhibitors are thus emerging as new modulators of protein function that could result in novel therapeutic applications. Bioluminescence Resonance Energy Transfer (BRET) is a powerful technology that exploits resonance energy transfer between a light-emitting enzyme (luciferase) and a fluorescent acceptor protein (YFP) to study PPIs. One partner protein is fused to the donor and the other to the acceptor, and if the two proteins interact and the distance between the donor and the acceptor is less than 10 nm, resonance energy transfer occurs and a light signal, corresponding to light reemission by the acceptor, can be detected. We set up yeast-BRET as a high-throughput platform (>800 compounds/day) for monitoring PPIs *in vivo* and for screening/identifying new potential PPI inhibitors. Key features of our technology are: i) the utilization of a hyper-permeable *S. cerevisiae* strain, rather than mammalian cells, as a screening platform allowing more flexibility with regards to assay optimization (e.g., different strains and growth conditions as well as inducible promoters); and ii) the use of a high-efficiency small donor luciferase (NanoLuc) instead of the *Renilla Luciferase* (Rluc), which results in a more efficient protein expression and signal intensity/stability. We validated and optimized yeast-BRET using the p53-HDM2 interaction and the inhibitors Nutlin-3 and Nutlin-3a. The use of different growth conditions, type of donor (Nanoluc or Rluc) and donor/acceptor ratio greatly impacted on assay sensitivity, confirming yeast as an excellent model organism for PPI inhibitor screenings. Following up to the results obtained from the study of the p53-HDM2 interaction, we further optimized and translated screening conditions to PPIs between viral nonstructural proteins and to the immune-modulating CD40/CD40L and 2B4/CD48 receptor-ligand interactions. An extracellular, surface-yeast BRET system is also being developed, in order to achieve expression of both partners as extracellular fusion proteins in a yeast display, protease-deficient, strain (EBY100). Both systems are being employed for the screening of >15,000 compounds from public libraries.

PS7-29: Investigation of broad range antiviral candidates in yeast virus system

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Saccharomyces cerevisiae for a long time has been a key model organism for the study of higher eukaryote-related processes, infectious diseases including. Double-stranded RNA viruses of *S.cerevisiae*, representatives of L-A and M families are widely distributed in nature. These dsRNAs encode a sole secreted protein, called killer toxin, empowering the cell to kill yeast lacking it or one carrying different killer type. L-A genome encodes the major structural protein Gag and Gag-Pol fusion protein, product of ribosomal frameshifting. Gag-Pol has transcriptase and replicase activities necessary for maintenance of both L-A and M satellite dsRNAs, so making self-sufficient system, directly linking polymerase activity to killer phenotype expression. For creation of

universal antiviral compounds targeting broad range of viral polymerases, we employed conjugate compound strategy. In general, small molecule inhibitor was coupled on to different positions of nucleotide, so resembling class of Nucleotide Reverse Transcriptase Inhibitors (NRTIs), common for treatment of HIV infection. However, we went further by linking catalytic event of nucleotide incorporation into nucleic acid with release of inhibitor. For testing of such compounds, approach of DNA biosynthesis *in vitro* was used first. It was designed to address incorporation of compounds of interest at single nucleotide resolution, relying on primer extension by purified DNA polymerases of choice. This system addresses both incorporation and/or resistance for it of nucleotide of interest, also enabling experiments on nucleobase selectivity of enzymes. Once promising candidates were selected, *in vivo* system targeting yeast virus has been elaborated. Double-stranded RNA-based viruses of *S. cerevisiae* are well known to be inherently meta-stable, leading to expelling of one of viruses (usually killer virus) due to the treatment of cells by factors such as elevated growth temperature and certain chemical agents (cycloheximide, etc). We hypothesised that presence of polymerase-targeting compound should act as similar factor, leading to expelling of killer virus from a cell and deprivation of the killer phenotype. Therefore, yeast strain bearing L-A and M system was used for evaluation of nucleotide-based compounds for ability to inhibit L-A-originated Gag-Pol fusion protein, barely investigated so far, aiming at the property of such compounds to eradicate killer virus from the parental strain.

PS7-30: Xylaria, a new source of potential antifungal agent

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A search for alternative sources of antibiotic agents has led to the discovery of new compounds with promising bioactivities against various medically important and food-borne microbial. A majority of antimicrobial agents are originated from natural products, including herbal plants and fungi. Due to the emergence of new multi-drug resistant fungal pathogens, it is important to better understand drug resistance mechanisms in order to find more effective drug targets. This study aims to identify some natural products with promising antifungal activities using yeast *Saccharomyces cerevisiae* as a model to test combinatorial inhibitory effects between different classes of clinical antifungal drugs and *Xylaria* extracts as a new approach to increase the drug efficacy and to reduce side effects. Here, we examined the potential of the fungal *Xylaria* to produce some active compounds with promising antifungal activity and investigated on the genes involved in drug resistance, namely *PDR5* transporter gene as well as some oxidative stress genes *YAP1/2* and *MSN2/4*. Deletion of these genes altered cell sensitivity to *Xylaria* extracts. Thus, we have identified new source for a potential antifungal agent from natural bioresources.

PS7-31: Modeling the process of human Alpha-Synuclein degradation in the cells of the thermotolerant yeast *Hansenula polymorpha*

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Parkinson's disease (PD) is characterized by selective loss of dopamine-producing neurons in *substantia nigra pars compacta*, and by the presence of inclusions called the Lewy bodies in which protein α -synuclein (α -syn) is the main constituent part. Various trigger factors, either genetic such as point mutations in α -syn encoding gene (*SNCA*) or environmental such as high temperature, metal cations (particularly Cu^{2+} , Fe^{2+} and Mn^{2+}) and pesticides can lead to misfolding, oligomerization or loss of normal function of α -syn resulting in development of neurodegeneration. Although some studies have suggested that the ubiquitine-proteasome system is the main for α -synuclein degradation, it was shown that such degradation is mainly carried out by lysosomal pathway, and in particular by macroautophagy and chaperone-mediated autophagy. We utilized the recombinant strains of the thermotolerant methylotrophic yeast *Hansenula polymorpha* for modeling the processes leading to PD in humans and for screening different factors that potentially induce α -syn aggregation (such as metal ions) and autophagic degradation (such as nutrient and amino acids deficiency). The fused ORF *SNCA-GFP* under P_{MET25} promoter was multicopy integrated into the genome of *Hansenula polymorpha* wild type strain (NCYC495) and

transformants with different copy number of *SNCA* expression cassette were isolated. We observed that human α -syn overexpression was toxic for the host cells. Our preliminary data indicate that nutrient limitation (nitrogen starvation) up-induced α -syn degradation in model yeast strains, whereas Mn²⁺ ions overexposure of these strains affected the process of α -syn degradation.

PS7-32: Screening for antifungal properties of medicinal wild plants of Northern Italy

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The risk of invasive fungal disease is increased in immune-compromised individuals, causing higher levels of mortality than tuberculosis or malaria. Antifungals frequently used in clinics to treat mycoses are essentially limited to the drug classes polyenes, echinocandins and azoles. The treatment with these established agents is often unsuccessful because of a series of limitations like nephrotoxicity, fungistatic mode of action and rapid development of drug resistance [1]. Hence, the need for new antifungals with fewer dose-limiting side effects, new mechanism of action and with a broad spectrum of antifungal activity is of prime importance. The plant kingdom has always represented a rich source of bioactive molecules. In recent years the research on natural products for the discovery of new drugs to be used directly or considered as a base for the development of better drugs is receiving a renovated interest [2]. In the present study we investigated the antifungal activity of ten medicinal wild plants of the Italian Trentino Alto-Adige region. Therefore, plant extracts have been tested against a broad panel of clinical isolates of human pathogenic fungi (*Candida albicans*, *C. parapsilosis*, *C. glabrata*, *C. lusitaniae*, *C. tropicalis*) using the broth micro-dilution assay. Extracts that yielded interesting antifungal activity (MIC50 >125 ug/ml) have been subjected to bioassay-guided fractionation. The data collected support the folkloric use of plants to treat diseases and skin infections.

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PS7-33: The impact of *PDR16* gene deletion in pathogenic and nonpathogenic yeast species

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Fungal infections pose a growing threat to human health as the population of immunocompromised patients grows. Among the quite small number of clinically effective antifungal drugs, azole antifungals are often the primary choice in treating fungal infections. However, yeast and fungi are able to develop resistance to counteract the action of azoles. One of the recently emerged factor of clinical azole resistance in human fungal pathogens is the product of yeast *PDR16* gene – Pdr16p. The loss of the *PDR16* gene function in different yeast species such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Candida albicans* and *Candida glabrata* leads to increased azole susceptibility, apparently due to an enhanced drug uptake by mutant cells. Pdr16p (also known as Sfh3) is a member of the yeast Sec14-like phosphatidylinositol transfer protein family. It facilitates transfer of phosphatidylinositol (PI) between membrane compartments in *in vitro* systems. The binding of PI to *ScPdr16p* represents an essential feature of the protein for providing protection against azole antifungals. It is not known yet, whether the role of Pdr16p in conferring resistance to azole antifungals is direct or mediated via some signaling role of Pdr16p. In the present study we assessed the role of the *PDR16* gene in the osmotolerance and halotolerance of *C. glabrata* and *K. lactis*. The *K. lactis* *pdr16Δ* deletion mutant displays a reduced ergosterol content, altered plasma membrane properties and hypersensitivity to alkali metal cations (Li⁺, Na⁺, K⁺) indicating the possible role of *KlPdr16p* in the maintenance of cellular ion homeostasis. The absence of *CgPdr16p* did not affect the response of *C. glabrata* to stresses induced either by hyperosmotic conditions or alkali metal cations. The response of the *PDR16* gene expression to the absence of transcription factors involved in regulation of multidrug resistance and oxidative stress response in both yeast species was also assessed. Our results indicate that *PDR16* gene belongs to genes whose expression is induced by chemical and oxidative stresses. The reduced halotolerance of the *K. lactis* *pdr16Δ* mutant strain points to the possible differences between the two yeast

species in the plasma membrane and/or cell wall structures.

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PS7- 34: A Genetic code alteration accelerates the acquisition of antifungal drug resistance in *Candida albicans*

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Fungal infections are an increasingly serious problem in light of advances in modern medical practices and immunosuppressive diseases. Resistance to frequently administered antifungal drugs, such as azoles, is steady increasing. In the human fungal pathogen *Candida albicans* the evolution of drug resistance is driven by phenotypic variability, its underlying DNA mutations and by a high degree of genomic plasticity. A particularity of *C. albicans* stress response repertoire is the ability to vary the levels of leucine and serine at CUG positions on a genome wide scale. Here we show that increased levels of mistranslation, like in bacteria, hasten the appearance of drug tolerance and resistance in the eukaryote *C. albicans* by accelerating genome diversification.

PS7-35: Toward elucidating exoribonuclease-dependent mechanisms for the toxicity of and resistance against the anti-cancer drug 5-Fluorouracil

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5-Fluorouracil (5-FU) has been widely used to treat solid tumors. The drug is known to inhibit enzymes involved in DNA replication (TK) and RNA processing/modification (Rrp6/EXOSC10). However, the molecular mechanism of 5-FU's anti-proliferative activity is not fully understood and 5-FU resistance is a serious clinical problem. We hypothesize that (i) long non-coding RNAs regulated by Rrp6/EXOSC10 are important for 5-FU toxicity and that (ii) elevated levels of the enzyme may confer 5-FU resistance in cancer cells. We compared the transcriptomes of a wild type yeast strain treated with 5-FU to an rrp6 deletion mutant and identified transcripts that respond to the drug, the RRP6 deletion or both. Furthermore, we discovered that RRP6 overexpression confers 5-FU resistance during vegetative growth. Our data offer interesting leads for the discovery of novel protein-coding and non-coding RNAs that may be involved in 5-FU toxicity, and they indicate that elevated levels of Rrp6 compromise 5-FU activity. These results are potentially important for improving 5-FU based chemotherapy.

Poster Session 8: Interplay and dynamic of organelles

PS8-1: Dual subcellular localization of Fad1p in *Saccharomyces cerevisiae*: A possible choice at post transcriptional level

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FAD synthase (EC 2.7.7.2) is the last enzyme in the pathway that converts riboflavin into FAD. In *Saccharomyces cerevisiae* the gene encoding for FAD synthase is *FAD1*, from which a sole protein product (Fad1p) is expected to be generated. Here we proved on molecular basis that a natural Fad1p exists in yeast mitochondria and that, in its recombinant form, the protein is able *per se* both to enter mitochondria and to be destined to cytosol. Thus, we propose that *FAD1* generates two echoforms, that means two identical proteins destined to different subcellular compartments. An analysis made of the 3'UTR of *FAD1* mRNA by 3'RACE experiments revealed the existence of (at least) two *FAD1* transcripts with different 3'UTRs, both containing a predictable *cis-acting* motif, which could be differently involved in mRNA degradation/protein destination. Comparing the carbon source dependence of the mitochondrial Fad1p level with that of the *FAD1* transcripts we propose that the longer transcript might favour the generation of mitochondrial Fad1p echoform.

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PS8-2: Mitochondrial import of peroxiredoxin Prx1p involves two cleavages by MPP and Oct1p

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Mitochondrial peroxiredoxin (Prx1p) from *Saccharomyces cerevisiae* is a thiol-dependent peroxidase involved in mitochondrial hydrogen peroxide reduction. Prx1p is a member of the 1-Cys Prx and the mechanism involved in reduction of cysteine-sulfenic acid (Cys-SOH) generated by the reaction with hydrogen peroxide is still poorly understood. Initially the mitochondrial thioredoxin system (composed by proteins Trx2p and Trx3p) and later on Grx2p were proposed to reduce Prx1p. Prx1p is synthesized in the cytosol as a precursor with a cleavable N-terminal targeting signal (presequence) and subsequently imported into mitochondria. The objective of this work is to elucidate molecular mechanisms of mitochondrial Prx1p import and submitochondrial localization. We initially found that Prx1p is localized in the matrix compartment in a soluble form through submitochondrial fractionation and western blot analysis. Trx2p and Trx3p were also localized in the same compartment, supporting a role of these proteins in Prx1p reduction. Furthermore, the importing process of Prx1p into mitochondrial matrix involves processing by octapeptidyl aminopeptidase 1 (Oct1p), which removes an octapeptide from the N-terminus of the Prx1p-intermediate, which is generated by mitochondrial processing peptidase (MPP). The vast majority of matrix-targeted proteins is cleaved by mitochondrial matrix processing peptidase (MPP), which removes the presequences. In contrast, Oct1p has a more specific subset of proteins. In order to assess the role of Prx1p cleavage by Oct1p, we compared the stability of Prx1p in wild-type and *ΔOCT1* mitochondria. We found that Oct1p cleavage increases the half-life of Prx1p. However, this cleavage does not significantly alter the peroxidase activity of Prx1p *in vitro*, as assessed by NADPH consumption assays using the recombinant proteins Trx2p, Trx3p and two isoforms of Prx1p (representing the forms cleaved and not-cleaved by Oct1p). Finally, we are currently identifying the MPP and Oct1p-cleavage sites at the Prx1p N-terminus by mass spectrometry using immune-precipitated Prx1p from wild-type and *ΔOCT1* mitochondria. Our results show that processing of Prx1p-intermediate pre-protein by Oct1p leads to a stabilization of Prx1p after import into the mitochondria. This constitutes a protein quality control system that regulates Prx1p homeostasis and probably mitochondrial redox processes.

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PS8-3: Intracellular sodium distribution in the halotolerant yeast *Debaryomyces hansenii*

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Sodium is the most abundant cation in natural environments and its accumulation is toxic for most organisms. Accordingly, the model yeast *Saccharomyces cerevisiae* has developed transport systems to exclude this cation from the cell and keep low amounts of sodium. Moreover the cation is not homogeneously distributed inside the cell and while vacuoles and nuclei retain most of intracellular sodium, the cytosolic fraction is virtually free of sodium in *Saccharomyces* wild type cells [1]. On the other hand, the halotolerant *Debaryomyces hansenii* keeps higher amounts of intracellular sodium than *S. cerevisiae* and it has been defined as a sodium includer yeast [2,3]. On the basis of the isolation of the main *Debaryomyces* organelles we have optimized a procedure to determine the subcellular location of sodium in cells grown under several NaCl concentrations. Our results show that sodium concentrations close to those present in *D. hansenii* natural habitats such as sea water induced osmotic stress but did not importantly affect growth. Under these conditions vacuoles play a regulatory function in cation homeostasis but the cytosolic fraction of this yeast contains relatively high amounts of sodium. We propose that while vacuoles adapt to regulate cation content playing an important role in general cation homeostasis, sodium is not a specifically toxic element in *D. hansenii*.

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PS8-4: Import of *Saccharomyces cerevisiae* Fox2p into peroxisomes depends on the novel, Non-PTS1 and Non-PTS2 targeting signal

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Peroxisomes are single-membrane organelles present in almost all eukaryotic cells. They are responsible for fatty acid beta-oxidation, H₂O₂ decomposition, cholesterol and aminoacids synthesis. Proteins are imported into the peroxisomal matrix posttranslationally and the specificity of transport is dependent on the targeting signal encoded in the sequence of the protein. Two such signals are known, PTS1 and PTS2, recognized by two import receptors, Pex5p and Pex7p, respectively. Yet in *S.cerevisiae* there are at least two proteins that either do not have any such signals (acyl-CoA oxidase, AOx) or that posses the PTS1 signal but do not need it to be efficiently imported (carnitine acetyl-CoA transferase, Cat2p). Their import depends on the N-terminal part of the Pex5p receptor, distinct from its PTS1-specific C-terminal region containing TPRs [Skoneczny, & Lazarow (1998) Mol Biol Cell 9S:348A; Distel et al. (2002) J Biol Chem. 277:25011]. To better characterize this novel mechanism of peroxisome-destined protein recognition we searched, using metal affinity chromatography, for new peroxisomal proteins that physically interact with the N-terminal region of Pex5p. We identified the Fox2p protein that has the PTS1 signal on its C-terminus, so it was believed to be imported via PTS1 route. Yet Fox2p with absent or non-functional PTS1 is still imported into peroxisomes, albeit less efficiently. In the absence of the PTS2 receptor Pex7p in the cells the import of Fox2p is not affected, whereas in the absence of Pex5p it is abolished completely. Taken together, we identified another protein whose import into peroxisomes at least partially depends on the novel, non-PTS1 and non-PTS2 signal and on the interaction with N-terminal Pex5p domain distinct from the PTS1-recognizing TPR region.

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PS8-5: Aim23 is an yeast mitochondrial translation initiation factor 3 which is unnecessary for protein synthesis

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Mitochondria are essential organelles of virtually all eukaryotic cells. They have their own genome and are able

to transcribe and translate their genetic material. The system of mitochondrial protein synthesis is organized in a manner close to that of prokaryotes. However, mitochondrial DNA contains just a few protein-coded genes (9 in yeast, 13 in humans), so the mitochondrial translation system deals with a limited number of mRNAs. The mitochondrial translation machinery is also somewhat lineage-specific, with various components being gained and lost in different taxonomic groups. The classical bacterial initiation factors (IFs) IF1, IF2 and IF3 are universal in prokaryotes, but only IF2 is universal in mitochondria (mIF2). No IF1 has been identified in mitochondria of any organism. An insertion in mIF2 has been suggested to functionally compensate for the absence of mIF1. Mitochondrial IF3 (mIF3), although known to be present in various eukaryotes, has not been identified for many years in budding yeast *Saccharomyces cerevisiae*, the model organism for studying mitochondrial translation *in vivo*. In 2012, we have proven that IF3 does present in yeast mitochondria, and it is Aim23 protein. In the present study, we have characterized the effects of AIM23 gene deletion on yeast mitochondrial function. One could suggest that such a deletion would lead to a complete loss of respiration, translation and other molecular processes in mitochondria. However, this was not the case: the growth of AIM23 Δ yeast on glycerol-containing media was suppressed in first 1-2 days only and reached the levels of wild-type in 3-4 days. AIM23 Δ cells also were able to respire. Interestingly, we observed a very unusual pattern of mitochondrially-synthesized proteins in the Δ AIM23 strain. The amount of several proteins is decreased in the mutants compared to the wild-type but the amount of some others is increased. We conclude that the yeast cells are able to adapt somehow to the absence of Aim23p.

PS8-6: The Q/N-rich protein kinase Sch9 as a modulator of nonsense suppression and a potential prion protein

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Yeast prions are heritable protein factors which have ability to maintain their modified structure during their transmission from mother cell to daughter cell. More than dozen yeast prions are known to date. Our laboratory discovered one of them, the [*ISP* $^+$] prion, which diminishes suppression of *lys2-87* and *his7-1* nonsense mutations caused by a *sup35* mutation. [*ISP* $^+$] is suggested to result from Sfp1 protein prionization. Sfp1 is a transcription factor that plays an important role in regulation of ribosome biogenesis. The protein kinase Sch9 is another significant factor regulating this process. Moreover, both regulators are the major targets of the TORC1 kinase. As yet the mechanism of [*ISP* $^+$] induction and maintenance remains unknown. Therefore, we were interested if Sch9 interacts with [*ISP* $^+$] aggregates or Sfp1. Interestingly, Sch9 is Q/N-rich like most known prion proteins. Hence, the question arises whether Sch9 can form a prion. We showed that plasmid-borne *SCH9* overexpression decreases suppression of some nonsense mutation in both [*isp* $^-$] and [*ISP* $^+$] strains, even so this phenotype disappears after plasmid loss. For the purpose of finding out if Sch9 forms prion-like aggregates we studied intracellular localization of an Sch9-YFP fusion in the yeast cells. We distinguished three types of fluorescence: diffuse, multiple of small dots and single dot. In order to find out if this pattern depends on [*ISP* $^+$] we investigated Sch9-YFP fluorescence in [*ISP* $^+$] and [*isp* $^-$] strains and found no difference. In addition, we identified three types of cells described above in the other strains, which means that the effect is not strain specific. Also we found out that fluorescence type of Sch9-YFP relies on growth phase: cells with diffuse fluorescence prevail in early logarithmic stage whereas cells with single dot in late logarithmic stage and in the stationary phase. This dynamics might be associated with vacuole development. In sum, our data may establish a link between *SCH9* and nonsense suppression, and overproduction of Sch9-YFP leads to different patterns of fluorescence. The authors acknowledge SPbU for research grants 1.37.291.2015, 1.41.546.2015 and RFBR for a grant 14-04-31265.

PS8-7: A genome-wide overexpression screen for mitophagy genes in yeast

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Significant contribution to the understanding of mitophagy has come from research in the model organism *Saccharomyces cerevisiae*. The results of two genetic screens of a non-essential gene deletion library have identified two genes, ATG32 and ATG33, encoding key components of the mitophagy pathway both localised at the outer mitochondrial membrane (OMM). Atg32p is phosphorylated by mitogen activated kinases allowing

interaction with core autophagy proteins resulting in mitophagy. However, our knowledge of mitophagy and its regulation in yeast is incomplete and requires further investigation. To this end, we have devised a high-throughput imaging assay based on the use of a mitochondrially targeted fluorescent protein biosensor called mt-Rosella. The assay is performed using 96-well format in a semi-automated fashion and allows the screening of different strain libraries under a range of conditions. We investigated the effects of gene overexpression on mitophagy using a library comprising 1588 high-copy number plasmids covering more than 95% of the yeast genome including essential genes (previously excluded from the deletion library). Each plasmid contains 4-5 contiguous genes each under the control of their native promoter. Results of the screen revealed that increased expression of a number of genes affects mitophagy. Among these, we found that OM14 expressing the OMM protein Om14p confers a strong decrease in mitophagy phenotype when overexpressed. Subsequently we found a similar phenotype when the gene was deleted. Om14p is required for co-translational import of proteins into mitochondria and it also forms a complex with OMM proteins Por1p and Om45p. We anticipate that increased or reduced amounts of Om14p could derange normal OMM architecture and indirectly affect proteins essential for mitophagy, including Atg32p. Further investigation is required to fully understand the role of Om14p in mitophagy.

PS8-8: Approaching the 3D structure of a fungal conserved hub protein

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Intrinsically disordered proteins (IDPs) are involved in numerous essential biological processes. Their conformational flexibility gives them the ability to be involved in one-to-many binding (where a single disordered domain is able to bind several structurally diverse partners). Remarkably, highly connected proteins located at nodes in interactions networks or “Hubs” are significantly enriched in disordered domains which allow them to fulfil their multiple interactions. Knr4/Sml1 is a *S. cerevisiae* hub protein, specific of the fungal kingdom, whose potential flexible structure enables interacting with several partners and ensuring functions in gene transcription, cell cycle progression and morphogenesis. In most *S. cerevisiae* genetic backgrounds, *KNR4* deletion impairs growth under stress conditions such as elevated temperature, presence of SDS, caffeine, or cell wall-affecting drugs. Over 280 synthetic lethal or sick interactions have been described, revealing the central role of Knr4 at a node position connecting several essential pathways. Structural secondary similarities analyses indicate that Knr4 might be related to distant gene products from the bacterial kingdom, and suggest that *KNR4* gene may have reached the eukaryote kingdom through viruses. However, the 3D structure of fungal Knr4 is unknown to date. *In silico* analysis, combined with biophysical and biochemical methods, has shown that it contains large disordered regions on the N-terminal (1-80) and the C-terminal (341-505) parts, while the central core, which holds the essential of the biological functions of the protein, appears structured and globular. The N and C terminal parts are involved in ensuring and controlling the interactions of Knr4 with its protein partners. Deciphering the structure of the central functional core represents the first step towards the achievement of obtaining the global 3D structure of the complete protein. We have expressed in *E. coli* and crystallized this core domain, as well as a modified Selenomethionine containing corresponding protein. We have identified secondary structure elements, and recently obtained new crystals diffracting at 2.5 Å which should allow us to rapidly decipher the 3D structure of the protein core of this unique fungal IDP.

PS8-9: Exploring the endocytic pathway by combining high-throughput genetics and high-content microscopy

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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 pathways. As a result, endocytosis impinges on a number of physiological processes, including cell movement, adhesion, growth and differentiation as well as pathogen virulence and drug delivery. In order to gain a global understanding of the function and molecular regulation of the endocytic pathway, we have combined synthetic genetic array (SGA) analysis with high-throughput confocal fluorescence microscopy and quantitative image analysis, and assessed 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). Moreover, we performed real-time fluorescence microscopy on a genome-wide scale to identify genetic factors affecting the spatial and temporal dynamics of endocytic vesicle formation from the plasma membrane. Our systematic and automated approach identified over 400 genes that affect either the morphology of the studied endocytic compartments or the dynamics of endocytic vesicle formation, many of which have not been directly associated with the endocytic process before, including some previously uncharacterised genes.

PS8-10: Mechanism for sensing lipid asymmetry of the plasma membrane and external alkalization

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In the eukaryotic plasma membrane (PM), lipid composition differs between the cytosolic and extracellular leaflets, which is called lipid asymmetry. For example, phosphatidylserine (PS) is mostly confined to the cytosolic leaflet, while sphingolipids are enriched in the extracellular leaflet. Lipid asymmetry is generated by inward (flip) and outward (flop) movement of the lipids between the leaflets. We previously reported that changes in lipid asymmetry are sensed by the PM protein Rim21 [1] in the Rim101 pathway, which was originally reported to detect external alkalization. The signal emitted by Rim21 is transduced at the PM [2]. However, the mechanism by which Rim21 senses lipid asymmetry and external alkalization remains unclear. To address this issue, we performed a detailed analysis of Rim21. We first focused on the C-terminal cytosolic region of Rim21 (Rim21C), where charged amino acid residues are highly enriched. Although Rim21C does not possess a transmembrane segment, GFP-Rim21C was primarily detected at the PM of WT cells. Interestingly, in mutants with disturbed lipid asymmetry, because of inactivation of the flip-flop movements of lipids, GFP-Rim21C was found to be dissociated from the PM. GFP-Rim21C also dissociated from the PM upon external alkalization. These observations indicate that Rim21C alone can sense alterations in lipid asymmetry and external pH, and responds to them through changing its affinity to the PM. In other words, a sensor motif exists in Rim21C. We identified a sensor motif composed of two adjacent clusters of charged amino acid residues, the ERKEE and EEE motifs, by using mutational analysis of Rim21C. The ERKEE motif, particularly the RK moiety, was shown to mediate the association of Rim21C with the PM and the EEE motif mediates its dissociation. Furthermore, lipid overlay analysis revealed that Rim21C binds to the negatively charged lipids, such as PS. Taken together, we propose an “antenna hypothesis” as a mechanism for sensing lipid asymmetry. In this hypothesis, Rim21 uses Rim21C like an insect antenna to monitor the state of lipid asymmetry through repetitive interactions with the PM by using the antagonistic ERKEE and EEE motifs.

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PS8-11: The involvement of FAD synthesis and trafficking in riboflavin-responsive human diseases: investigations in *S. cerevisiae* and *C. elegans*

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The vitamin B₂ or riboflavin (Rf), which in mammals necessarily derives from the diet, is converted through the

action of Rf kinase to FMN, which in turn, is metabolized to FAD by FAD synthase (FADS, EC 2.7.7.2) [1]. FADS, coded by *FLAD1* gene located on chromosome 1 in humans, is a ubiquitous enzyme, whose isoforms are localized in cytosol, mitochondrion and nucleus [2]. FADS plays a key role in the metabolic pathway that converts Rf into FAD, the redox co-factor of a large number of dehydrogenases, reductases and oxidases. Besides synthesize the cofactor, FADS was proven also to act as a sort of FAD chaperone during client flavoenzyme biogenesis [3]. Since many years we proposed that aberrant flavin cofactor metabolism is responsible for some cases of MADD (multiple acyl-CoA dehydrogenase deficiency, OMIM #231680), in particular those responding to high-doses of Rf treatment [4]. Somehow related to this pathology is the Brown-Vialetto-van Laere syndrome (OMIM #211530; #614707) a rare neurological disease in which the functionality of Rf translocators (SLC52A3; SLC52A2) is altered. In order to mimic at the organism level the molecular defects underlying Rf-responsive human pathologies, we introduced two models: *Saccharomyces cerevisiae* strains lacking of the mitochondrial FAD transporter gene, namely *FLX1* and *Caenorhabditis elegans* strains in which RNA interference was used to silence the single copy gene of *flad-1* gene, coding for different FADS isoforms. In both organisms the effects of altering flavin homeostasis on mitochondrial bioenergetics, ATP and ROS levels, and certain flavoenzyme activities/expression level were assessed in the frame of mitochondrial related phenotypical changes. The molecular rationale for Rf therapy will be also deal with in these systems.

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PS8-12: Genotypic identification of yeast isolated from Yagnobi fermented milk

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The Yagnob Valley in Tajikistan is inhabited by a small isolated human population. The area is geographically isolated, causing the inhabitant's culture, food and lifestyle to remain uninfluenced by the rest of the world. Traditional methods of fermenting milk involve the use of indigenous microorganisms, leading to the production of a variety of fermented milk products. The people in Yagnobi produce a fermented milk from goat, which constitutes one of their main foods. The aim of the current work was to determine which yeasts were present in the original Yagnobi fermented milk as well as in the same product reproduced at home for three years with monthly reculturing in cow milk. Thirty yeasts have been isolated using different lab media such as M17, MRS, WL, YPD and YPD plus chloramphenicol, and colonies were re-cultivated until pure. Twenty isolates have been obtained from the original product and 10 from fermented milk reproduced at home. They were identified by Sanger sequencing of the amplified ribosomal Internal Transcribed Spacers (ITS1-4). Furthermore, RFLP analyses of the ITS1-4 region were performed when quality, coverage or similarity of ITS1-4 sequences did not permitted the unambiguously identification of the yeast isolates. Only for *Saccharomyces cerevisiae*, identity was also confirmed with GTG₅Rep PCR. The results showed the presence of *Kluyveromyces marxianus* (11), *Pichia fermentans* (7) and *Saccharomyces cerevisiae* (10) in both products. However, *Kluyveromyces lactis* (1) was found in the original fermented milk sample and *Kazachstania unispora* (1) in home maintained fermented milk. Great attention has nowadays been devoted to microbial resources and the study of traditional fermented products can be a very interesting source of microbial biodiversity. This study provides for the first time data on yeast composition and characteristics in naturally Yagnobi fermented milk, showing an unexpected richness in yeast communities leading to fermented milk production.

PS8-13: Quest for new proteins imported into the peroxisomal matrix via the non-PTS1 and non-PTS2 pathway in *Saccharomyces cerevisiae*

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Peroxisomes are organelles with multiple functions in eukaryotic cells. In yeast *Saccharomyces cerevisiae*, peroxisomes are involved in fatty acid degradation and detoxification of reactive oxygen species. These compartments do not contain DNA or ribosomes, so peroxisomal proteins must be imported from cytosol. There are two known peroxisomal targeting signals: carboxy-terminal PTS1 found in a majority of peroxisomal matrix proteins and amino-terminal PTS2. Import machinery consists of several membrane and cytosolic proteins called peroxins. The PTS1 import pathway is dependant on a shuttling receptor - peroxin 5 (Pex5p), which binds proteins in cytosol and translocates them to the docking complexes at peroxisome membranes. Interestingly, some proteins, such as acyl-CoA oxidase (AOx), do not have PTS1 or PTS2 and yet are imported into the peroxisome matrix. Another example is carnitine acetyltransferase (Cat2p), which contains PTS1, but this targeting sequence is not necessary for proper import of Cat2p into peroxisomes. It was shown that AOx and Cat2p are also bound by Pex5p, but this interaction involves N-terminal region of Pex5p, different from that involved in PTS1 protein recognition (Skoneczny M, Lazarow PB., 1998. *Mol. Biol. Cell*, **9**S and Distel B., 2002. *J Biol Chem.* **277**). These results led to a conclusion, that Pex5p may additionally play a role in recognition of a yet to be discovered new peroxisomal targeting signal. Apart from AOx and Cat2p, there are other peroxisomal matrix proteins that possess no known PTS signals or proteins that are related by some means to peroxisomes, but their intracellular localization was not thoroughly determined. In our study, by using GFP tagging and fluorescence microscopy we examined this group of proteins to look for those that could localize to peroxisomes. Here we document two novel candidate proteins for non PTS1/PTS2 import route: a malate dehydrogenase 2 (Mdh2p) and catalase (Cta1p). Mdh2p does not contain PTS1 or PTS2 however in our experiments Mdh2p-GFP co-localized with peroxisomes. For Cta1p-GFP devoid of putative PTS1, peroxisomal localization was still observed. Our results indicate that the new hypothetical peroxisomal import route may be important for the translocation of numerous proteins. Funding: Polish National Science Center grant no.: 2013/08/M/NZ3/01028.

PS8-14: Cytokinesis after prolonged mitosis requires lipolysis of storage neutral lipid in budding yeast

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Neutral lipids are stored in the membrane-bound organelle lipid droplet (LD) in essentially all eukaryotic cells. The storage neutral lipids are thought to provide membrane and energy sources. However, it remains elusive what physiological conditions require the mobilization and utilization of these lipids. We have examined the quadruple deletion mutant *are1 are2 dga1 lro1* that lacks LD due to its inability to synthesize the neutral lipids sterol ester (SE) and triacylglyceride (TAG). We reveal that the quadruple mutant is delayed at the late stage of the cell division cycle. Biochemical and cytological analysis of cells released from mitotic arrest shows that the mutant is delayed at cytokinesis, but not at the onset or the progression of anaphase. The assembly and function of the cytokinesis machinery actomyosin ring, which constricts the mother-bud neck, appears to be intact in the mutant. Interestingly, the exocyst complex, which transiently mediates vesicle fusion at the plasma membrane for the deposition of membrane and proteins at the cytokinesis site, persists at the bud neck in the mutant. Furthermore, cytokinesis is also delayed in the *are1 are2* and *dga1 lro1* double mutants that are deficient in SE and TAG, respectively, indicating that both SE and TAG are important for cytokinesis after mitotic arrest. In addition, cells lacking the major TAG lipases Tgl3 and Tgl4 are defective in cytokinesis, indicating that lipolysis of TAG facilitates its utilization in the cell cycle. Membrane normally expands during mitosis in order to achieve organelle inheritance during cell division. We propose that this process primarily utilizes neutral lipids as its source. The LD-deficient mutant may sought other lipid reserve for membrane expansion. In return, lipid homeostasis is altered and ultimately perturbs the proper timing and localization of vesicle fusion during cytokinesis.

Poster Session 9: Autophagy and intracellular trafficking**PS9-1: Moderate overexpression of SEC16 improves α -Amylase secretion in *Saccharomyces cerevisiae*****Jichen Bao¹, Mingtao Huang¹, Dina Petranovic¹, Jens Nielsen^{1,2}**¹*Novo Nordisk Foundation Center for Biosustainability, Department of Biology and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden;* ²*Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark*

There is a large and increasing demand of recombinant proteins, not only for pharmaceuticals but also in the field of industrial enzymes. Recombinant proteins can be produced by a range of different hosts, including mammalian cells, insect cells, bacteria, yeasts and fungi. Each of these have their own advantages and disadvantages, and none would naturally make a suitable general platform for producing of a wide range of recombinant proteins, with satisfactory yield, titer and productivity. Therefore, host optimization is required for the development of efficient recombinant protein producers. Yeast *Saccharomyces cerevisiae* is one of preferred model microbial and eukaryal systems, because of its robustness, well-studied genetics and physiology, developed molecular tools and large free databases. The limitations sometimes include translation and sometimes the folding and secretory capacity, which is more challenging to address. In this study, we focused on the secretory pathway and improved the secretion of a model enzyme α -amylase by overexpressing *SAR1*, which is the trigger of COPII vesicle formation, and *SEC2*, *SEC4*, *SEC15* and *YPT32*, which are required for Golgi-derived vesicle budding and transport. When these secretory proteins were overexpressed individually from a low copy number plasmid with the strong constitutive promoter TEF, only *SEC4* overexpression strain showed ~20% improvement in the final titer of α -amylase. The result indicates that overexpression of secretion-related proteins individually might not have a huge improvement on the production of α -amylase, so we will focus on the combination of these secretion-related proteins.

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PS9-2: Genetic control of the inactivation and degradation of the cytosolic proteins in methylotrophic yeast**Nina V. Bulbotka¹, Kateryna O. Levkiv¹, Olena V. Dmytruk¹, Andriy A. Sibirny^{1,2}**¹*Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, 79005 Lviv, Ukraine;* ²*Department of Biotechnology and Microbiology, Rzeszow University, Zelwerowicza 4, Rzeszow 35-601 Poland*

Methylotrophic yeasts are capable to metabolize one-carbon compound methanol as sole carbon and energy source. Many enzymes of methanol metabolism are located in peroxisomes whereas some of them are of a cytosolic localization. Shift of methanol-grown cells into a glucose-containing medium leads to fast inactivation of peroxisomal and cytosolic enzymes of methanol metabolism. Inactivation of peroxisomal enzymes occurs due to the autophagic degradation (pexophagy) whereas mechanisms of the inactivation of cytosolic enzymes like fructose-1,6-bisphosphatase (FBPase), formaldehyde and formate dehydrogenases remain unknown. In baker's yeast, the catabolite degradation of FBPase occurs after shift of glucose-starved cells into a glucose-containing medium. It was shown that FBPase is degraded by the proteasome-dependent pathway after glucose starvation of the yeasts for 1 day and by the vacuole-dependent pathway (autophagy) after glucose starvation of the cells for 3 days. We studied mechanisms of FBPase degradation in methylotrophic yeasts. The wild type strain of *Pichia pastoris* GS200, the protease-deficient strain SMD1163 (*pep4*, *prb1*) and the strain with deletion of a gene of a glucose sensor, Gss1p were used in this research. FBPase activity and protein amount was studied after shift of methanol-grown cells into a glucose medium with proteasome inhibitor MG132 and without it. Substantial decrease of the specific activity of FBPase in the wild-type strain and strain defective in vacuolar proteases and the minor change of the activity in the $\Delta gss1$ strain in the cells without the inhibitor was observed. We also compared the FBPase activity of the strains defected in autophagy pathway ($\Delta mon1$, $\Delta ypt7$, $\Delta ccz1$) with the wild type strain of *P. pastoris*. The results of Western blot analysis showed decrease in FBPase quantity in the GS200 strain and the minor decrease this protein in the SMD1163 strain after transfer of cells from methanol medium in

a glucose containing medium not depending on duration of a glucose starvation. The quantity of this enzyme changes little in the $\Delta gss1$ strain indicating the need of glucose sensing for FBPase degradation. From the received results we can make a preliminary conclusion that two different ways of FBP degradation, proteasomal and vacuole-dependent, can occur in methylotrophic yeasts *P. pastoris*.

PS9-3: A genetic interaction network required to sustain fatty acid overload in yeast

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The synthesis of lipids is an evolutionary conserved and highly regulated process. An unbalanced total cellular lipid content is associated with several human disorders, such as obesity or type 2 diabetes. The first and rate-limiting step of fatty acid (FA) de novo production is catalyzed by the enzyme acetyl-CoA carboxylase (Acc1), which is inactivated by phosphorylation by AMPK/Snfl. Lack of Acc1 phosphorylation by Snfl results in FA overproduction and triacylglycerol accumulation in cytosolic lipid droplets. In this study, we used a synthetic genetic array (SGA) approach to identify genetic interactions in a mutant strain lacking the Snfl phosphorylation site in Acc1. We found that many components of autophagy complexes are over-represented in the genetic interaction map of the obese yeast strain, suggesting a functional link between autophagy and lipid homeostasis. Additionally, phospholipid (PL) remodeling and synthesis of PL via the Kennedy Pathway play an important role in maintaining lipid homeostasis under endogenous FA overload conditions. These results reveal novel processes and components that are crucial for cells to respond to deregulated FA/lipid metabolism.

PS9-4: The key factors of mRNA localization mechanism in mating process in yeast

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Formation of diploid cells in budding yeast calls a mating process. It occurs when two haploid cells with opposite phenotypes "a" and "alpha" grow up in an asymmetric fashion to each other and fused. This process is activated by "a" and "alpha" pheromone factors that are expressed and secreted by haploid cells. The mechanism of "alpha" pheromone (MFA1/2) synthesis occurs through an ER -Golgi pathway by alpha haploid cells. "Alpha" pheromone is transported and secreted from the cell by secretory vesicles. Recently we discover that pheromone "a"(MFA1/2)expression dependent on mechanism of mRNA localization. mRNA is delivered to a mating projections (shmoo),locally translated and secreted from the a cells. In this work we found regulatory factors of MFA1/2 mRNA localization and expression. We show that actin cytoskeleton and ER play an important role in this process. The malfunction of one of regulatory factor affected "a" factor expression, progression of mating process and lead to mating sterility in yeast.

PS9-5: Evidence for a non-endosomal function of the ESCRT-III like protein Chm7 at the endoplasmic reticulum

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Endosomal sorting complex required for transport (ESCRT) proteins are involved in a number of cellular processes, like endosomal protein sorting, HIV-budding, cytokinesis and plasma membrane repair. Here, we explored the function of a non-canonical member of the ESCRT-III protein family, the *Saccharomyces cerevisiae* orthologue of human CHMP7. Very little is known about this protein. *In silico* analysis predicted that Chm7 (yeast ORF *YJL049w*) is a fusion of an ESCRT-II and ESCRT-III like domain, which would suggest a role in endosomal protein sorting. However, our data argue against a role of Chm7 in endosomal protein sorting. The endocytic cargo protein Ste6 was not stabilized in a $\Delta chm7$ mutant and Chm7 responded very differently to a loss in Vps4 function compared to a canonical ESCRT-III protein. Instead, we present evidence that Chm7 localizes to the endoplasmic reticulum. In line with a function at the ER, we observed a strong negative genetic interaction between the deletion of a gene function (*APQ12*) implicated in nuclear pore complex assembly and

mRNA export and the *CHM7* deletion. Yeast 2-hybrid interactions were detected with other ESCRT-III proteins (Ist1, Snf7 and Vps2) and with Vta1/LIP5. This raises the possibility that Chm7 performs a novel function at the ER as part of an alternative ESCRT-III like complex.

PS9-6: Ectopic activation of cell wall integrity MAP kinase pathway from endosomal compartments in *Saccharomyces cerevisiae* upon PtdIns(4,5)P₂ depletion

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Class I phosphatidylinositol 3-kinases (PI3K) catalyze the conversion of PtdIns(4,5)P₂ into PtdIns(3,4,5)P₃. In mammalian cells, this second messenger controls important functions, like cellular proliferation and inhibition of apoptosis; in fact, the hyperactivation of this protein is commonly observed in cancer. The model yeast *Saccharomyces cerevisiae* constitutively lacks of class I PI3K activity. Heterologous expression of hyperactive versions of this protein in yeast leads to growth inhibition due to the depletion of the essential plasma membrane pool of PtdIns(4,5)P₂. Therefore, this yeast model has demonstrated to be useful for applied purposes such as the screening of PI3K inhibitors, but also provides a way to study the roles of PtdIns(4,5)P₂ in the yeast cell. PI3K expression caused several defects in vacuolar morphology, endocytic trafficking and polarized exocytosis when expressed in yeast cells. Time-course analyses revealed that the loss of PtdIns(4,5)P₂ from the plasma membrane correlates with both cell wall integrity (CWI) MAPK activation and actin depolarization. In fact, PI3K but not a kinase-dead mutant version, triggered the phosphorylation of the CWI MAPK, Slt2, as well as the expression of a typical CWI transcriptional reporter, *MLP1*. Consistently, PI3K expression in *S. cerevisiae* led to a global transcriptional profile reminiscent of that of cell wall stress conditions. Interestingly, Pkc1, the yeast orthologue of mammalian protein kinase C, which operates upstream the CWI pathway, was abnormally located in intracellular compartments that were associated to post-Golgi recycling endosomes. We propose an ectopic activation of the CWI pathway from recycling endosomes as a consequence of the loss of plasma membrane identity by PI3K-driven depletion of PtdIns(4,5)P₂.

PS9-7: Conformational rearrangements in a yeast killer toxin during host cell intoxication

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K28 is a virus encoded A/B protein toxin secreted by *Saccharomyces cerevisiae* that enters susceptible target cells by receptor-mediated endocytosis. After retrograde transport through the secretory pathway, the α/β heterodimeric toxin reaches the cytosol where the cytotoxic α-subunit dissociates from β, subsequently enters the nucleus and kills cells by blocking DNA synthesis and arresting cells at the G1/S boundary of the cell cycle [1]. The major focus of the present study was to dissect the molecular mechanism(s) of ER-to-cytosol toxin transport and to identify cellular components involved in this process. As member of the A/B toxin family, K28 contains a single disulfide bond (S-S) covalently connecting the cytotoxic A/α subunit with its cell binding B/β moiety. During host cell intoxication, the intermolecular disulfide is rearranged and finally cleaved, however the underlying mechanism(s) of S-S rearrangement and reduction is still poorly understood [2]. Yeast strains solely expressing the α' domain of protein disulfide isomerase (Pdi1p) in a pdi1-delta background are toxin resistant, indicating the involvement of Pdi1p in host cell killing. Mutant Pdi1p variants containing Cys-to-Ser substitutions in all active site cysteines are incapable to complement the K28 resistant phenotype, while K28 sensitivity is fully restored after expression of Pdi1p containing two cysteines in each of its two CXXC motifs. However, Pdi1p is unlikely to act as reductase as it is incapable to reduce a K28 heterodimer in which all three cysteines in B/β had been destroyed. Based on our findings we propose a model in which conformational and/or redox changes in K28 depend on pH changes during intracellular toxin transport that are in vivo prevented by the isomerase activity of Pdi1p.

Kindly supported by the Deutsche Forschungsgemeinschaft (SPP1710, SFB1027). [1] Carroll SY, Stirling PC, et al. (2009). *Dev Cell* 17, 552-60; [2] Suzuki Y, Schmitt MJ (2015). *Biol Chem.* 396, 539-54.

PS9-8: The Vps13 protein is involved in endocytic internalization and endosomal trafficking events in yeast

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Vps13 proteins are highly conserved in eukaryotic cells. In human there are four *VPS13* genes and mutations in two of them – *VPS13A* and *VPS13B* cause the rare hereditary disorders: Chorea-acanthocytosis (ChAc) and Cohen syndrome (CS), respectively. Red blood cells from ChAc patients display depolarization of cortical actin cytoskeleton and an interaction between VPS13A and β-actin has been shown. Mutations in *VPS13B* result in disintegration of the Golgi apparatus and impair the glycosylation of proteins in CS patients. In yeast there is a single version of *VPS13*. Yeast Vps13 was first identified as a protein involved in a delivery of a luminal protease to the vacuole. However, the molecular function of VPS13 proteins is still unknown. We have shown that null mutant *vps13Δ* displays defects in the organization of the actin cytoskeleton indicating that in yeast Vps13 may regulate actin polymerization or depolymerisation. Actin cytoskeleton is necessary to many cellular processes, so the involvement of Vps13 in various transport pathways was analyzed. The retrograde transport from Golgi to ER was shown to be intact in *vps13Δ*. In contrast, the *vps13Δ* mutant exhibited a defect in fluid-phase endocytosis and in removal of arginine permease Can1 from the plasma membrane. Furthermore, the plasma membrane lifetime of actin cytoskeleton protein markers involved in endocytosis like Las17, Abp1 and Sac6 were shorter and the behavior of some of the patch protein was also abnormal in *vps13Δ*. Kymographs representing movement of Las17 and Abp1 patches revealed that the non-motile phase of endocytosis was affected in *vps13Δ*. Lack of Vps13 also affected intracellular vesicle trafficking and disturbed the recycling of proteins back to the plasma membrane demonstrated using the reporter GFP-Snc1-Suc2 fusion protein. Finally, we have also shown that Vps13 also participates in sorting of the reporter protein in the multivesicular body. These results show that Vps13 is involved in endocytosis and regulates this process at multiple stages including both endocytic internalization and intracellular events.

PS9-9: Identification of regions in monocarboxylic acid transporter Jen1 requisite for its glucose-induced degradation and recognition by arrestin-related protein Rod1

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In *Saccharomyces cerevisiae*, import of pyruvate and lactate into cells is mediated by the plasma membrane transporter Jen1, whose expression and localization are tightly regulated by glucose availability. Upon a depletion of glucose, its expression is derepressed and it localizes at the plasma membrane. Re-addition of glucose to the medium provokes a rapid degradation of Jen1 through the ubiquitin-mediated endocytosis. An E3 ubiquitin ligase Rsp5 is responsible for ubiquitination of the plasma membrane transporters and requires arrestin-like adaptor proteins for recognition of target proteins. In the case of the glucose-induced inactivation of Jen1, Rod1 fulfills its role as an adaptor protein, and its activity is regulated by a phosphorylation/dephosphorylation cycle under the control of the glucose-signaling pathway. Although Rsp5 adaptors are thought to directly interact with target transporters, it is unclear how Rod1 recognizes Jen1 in response to glucose replenishment. In order to address this issue, we first identified regions required for endocytosis of Jen1. It is predicted that Jen1 has 12 transmembrane domains and its N- and C-termini are faced to the cytoplasm. Deletion and mutational analyses revealed that acidic amino acid motifs in both its N- and C-terminal tails were required for the degradation and endocytosis of mutants. Particularly, the C-terminal motif was important for the Rod1 dependent endocytosis. To analyze the interaction between Rod1 and Jen1, we utilized the bimolecular fluorescent complementation assay. After an addition of glucose, the fluorescence complementation was observed as multiple dots on the plasma membrane in the wild type cells. In the cells deleted with *REG1*, encoding a regulatory subunit of type 1 protein phosphatase Glc7 responsible for Rod1 dephosphorylation, this complementation was lost. These results suggested that the dephosphorylation of Rod1 promoted its association with Jen1. Moreover, the mutation in the acidic motifs of Jen1 significantly decreased the interaction between Rod1 and Jen1, suggesting that Rod1 may recognize these motifs of Jen1 to be recruited to Jen1.

PS9-10: Using yeast to screen for drugs for the treatment of inherited Parkinson's disease**Katherine Strynatka**, Pak Poon, Chris McMaster*Dalhousie University, Canada*

Current therapies for Parkinson's disease (PD) are limited to managing signs and symptoms; there is no treatment available that prevents or significantly delays progression of the disease. Approximately 10% of PD cases are inherited forms of the disease. Specifically, mutations in the kinase LRRK2 have been shown to cause PD. Previous studies showed that LRRK2 kinase activity is regulated by ArfGAP1 and that decreasing ArfGAP1 expression results in a decrease in toxicity of mutant LRRK2. Therefore, small molecules that inhibit ArfGAP1 may be a potential therapy for PD. To that end, we screened for compounds that inhibit human ArfGAP1. We expressed human ArfGAP1 under the control of a titratable promoter in the yeast *Saccharomyces cerevisiae*. Expression of human ArfGAP1 in yeast proved toxic to the cell. This toxic phenotype was exploited to perform a high-throughput small-molecule screen for compounds that inhibit ArfGAP1, which would restore viability to the cell. A panel of small molecules was screened, including 5,000 pharmacologically active compounds and off-patent FDA-approved drugs, and 100,000 novel small molecules. Six compounds were identified as potential inhibitors of ArfGAP1, all with a similar core structure suggestive of inhibition of the same target. These are being tested for their capacity to inhibit ArfGAP1 directly, and reduce LRRK2 toxicity in vitro and in vivo.

PS9-11: Overexpression of native *Saccharomyces cerevisiae* SNARE genes increased heterologous cellulase secretion**John Henry D. Van Zyl¹**, Riaan Den Haan², Willem H. Van Zyl¹¹*Department of Microbiology, Stellenbosch University, Stellenbosch 7602, South Africa;* ²*Department of Biotechnology, University of the Western Cape, Bellville 7530, South Africa*

SNAREs (soluble N-ethylmaleimide-sensitive factor attachment receptor proteins) are essential components of the yeast protein trafficking machinery and are required at the majority of membrane and vesicle fusion events in the cell [1]. A major obstacle to the successful utilization of *Saccharomyces cerevisiae* for the single-step hydrolysis and fermentation of cellulosic material to second generation bio-ethanol (consolidated bio-processing) remains its inferior yields for heterologous cellulases. We have demonstrated an increase in secretory titers for the *Talaromyces emersonii* Cel7A (a cellobiohydrolase) and the *Saccharomyces fibuligera* Cel3A (a β -glucosidase) expressed in *Saccharomyces cerevisiae* through single and co-overexpression of some of the ER-to-Golgi SNAREs (*BOS1*, *BET1*, *SEC22* and *SED5*). Overexpression of *SED5* yielded the biggest improvements for both of the cellulolytic reporter proteins tested, with maximum increases of 22% for the *Sf*-Cel3A and 68% for the *Te*-Cel7A. Co-overexpression of the ER-to-Golgi SNAREs yielded proportionately smaller increases for the *Te*-Cel7A (46%), with the *Sf*-Cel3A yielding no improvement. Co-overexpression of the most promising exocytic SNARE components identified in literature [2] for secretory enhancement of the cellulolytic proteins tested (*SSO1* for *Sf*-Cel3A and *SNC1* for *Te*-Cel7A) with the most effective ER-to-Golgi SNARE components identified in this study (*SED5* for both *Sf*-Cel3A and *Te*-Cel7A) yielded variable results, with *Sf*-Cel3A improved by 130% and *Te*-Cel7A yielding no improvement. Improvements were largely independent of gene dosage, with episomal variance between the most improved strains shown to be insignificant. This study has added further credence to the notion that SNARE proteins fulfil an essential role within a larger cascade of secretory machinery components that could contribute significantly to future improvements to *Saccharomyces cerevisiae* as protein production host.

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PS9-12: The role of the signal peptidase complex on the recognition of translocating polypeptides**Chewon Yim, Hyun Kim***School of Biological Sciences, College of Natural Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 151-747, South Korea*

The Sec61 translocon accommodates ER-targeted polypeptides including membrane proteins and secretory

proteins in its pore for their ER translocation. Membrane proteins are targeted to the endoplasmic reticulum (ER) by the first hydrophobic transmembrane (TM) segment whereas secretory proteins have an N-terminal signal sequence for ER targeting. Although TM domains are generally more hydrophobic than signal sequences, they share similar sequence context and are laterally released from the Sec translocon. The conventional view on the translocation event has not distinguished these two different types of sequences in much detail. A cleavable N-terminal signal sequence is one of the distinctive features of secretory proteins. When polypeptides enter the ER lumen through the pore of the Sec61 translocon, the signal peptidase complex (SPC) recognizes and cleaves the signal sequence. The translocon, however, also accommodates a great number of transmembrane segments of membrane proteins, most of which are not cleaved. That is, while signal sequences are cleaved by the SPC, TM domains evade the cleavage. Hence, it is assumed that the SPC distinguishes a cleavable N-terminal signal sequence from a signal anchor sequence, the underlying mechanism of which is unknown. This study aims to elucidate the key players involved in the recognition, selection, and discrimination of ER-targeted polypeptides. We recently observed that model membrane proteins with a putative SPC-mediated cleavage site were more efficiently cleaved in the absence of Spc1p or Spc2p, the non-essential subunits of the SPC. The degree of cleavage efficiency differed dependent on the hydrophobicity of the transmembrane domain harboring the cleavage site. Based on the assumption that translocating nascent chains must be either recognized for or discriminated from being subjected to the SPC cleavage activity, these data suggest that Spc1p/Spc2p may be involved in the regulation of the recognition of the substrates for proper processing by the SPC.

PS9-13: Non-selective autophagy induced by phosphate starvation requires Atg11 and Is regulated by TORC1 signaling pathway in *Saccharomyces cerevisiae*

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Macroautophagy (hereafter autophagy) is a membrane-traffic pathway responsible for degradation of intracellular components including organelles and aberrant proteins. This catabolic process is primarily induced by nutrient starvation in which its degraded materials are recycled for survival. In *Saccharomyces cerevisiae*, it is known that a depletion of various nutrients such as nitrogen, carbon, and sulfur sources induces autophagy. As for phosphate, it is unclear that its depletion provokes autophagy, although phosphorus is an essential nutrient for all organisms. In order to analyze phosphate starvation-induced autophagy (PSiA), we expressed CFP fused with a peptide derived from a multicloning site of pRS416 (designated as CFP*). Upon delivery to the vacuole, the peptide is removed from CFP* in an autophagy-dependent manner, which allowed us to use it to monitor a non-selective bulk autophagy. Using this substrate, we found that autophagy was induced by phosphate starvation, but it was at lower level than that in nitrogen starvation. Deletion of *PHO91* gene, which encodes a vacuolar transporter exporting inorganic phosphate from the vacuolar lumen to the cytoplasm, activated PSiA, suggesting that the cytoplasmic phosphate level was sensed for autophagy induction. However, PSiA was not modulated by the *PHO* regulatory pathway, which induces an expression of genes related to phosphate assimilation, but rather by target of rapamycin complex 1 (TORC1) signaling pathway as in nitrogen starvation-induced autophagy. We also found that PSiA required Atg11, an adaptor protein for cargo recognition in selective autophagy, although it is dispensable for nitrogen starvation-induced autophagy. Moreover, Atg11 mutant protein lacking the cargo recognition domain was enough to induce PSiA. These results suggested that Atg11 was involved in autophagosome formation as well as cargo selection.

PS9-14: Toxicity of human Nedd4 ubiquitin ligase in yeast depends on Atg1, Atg14 and Atg18 autophagy related proteins

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Rsp5 ubiquitin ligase is a unique yeast member of the Nedd4 family of proteins. Human Nedd4 ubiquitin ligase or its variants, ectopically expressed, inhibit yeast cell growth by disturbing the actin cytoskeleton organization and dynamics [1]. In a screen for multicopy suppressors, which restore growth of *NEDD4w4*-expressing yeast

cells, we found the central fragment of *ATG2* gene encoding a core autophagy protein. Similarly, fragment of *ATG2* encoding part of Atg2, which contains APT1 and ATG-C domain, Atg2-C1, improved growth and actin cytoskeleton organization and dynamics of *NEDD4w4*-expressing cells. However, full-length *ATG2* was not effective. GFP-Atg2 protein in wild type cells is located in phagophore assembly site (PAS) and in a few small cytoplasmic punctate structures. The GFP-Atg2-C1 protein in Nedd4w4-expressing cells localizes to one punctual structure adjacent to the vacuole. This localization was not affected in several *atg* deletion mutants, suggesting that it might not be a PAS. Mutations *atg1Δ*, *atg14Δ* and *atg18Δ*, but not *atg9Δ* or *atg13Δ*, suppressed growth defect of Nedd4w4-producing cells. GFP-Atg2-C1 punctual structure in Nedd4w4-producing cells was surrounded by actin filament ring. The *atg18Δ* mutant devoid of Atg18 lipid-binding protein greatly affected its shape observed in a confocal microscope. Production of Nedd4w4 increased the cellular level of ubiquitinated proteins in yeast cells. GFP-Atg2-C1 had an opposite effect. These results suggest that Nedd4 ubiquitinates proteins in yeast, most probably the Rsp5 substrates, and together with Atg2 and other Atg proteins affects the formation of perivacuolar structure not identical to the PAS, which may be involved in protein degradation.

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Poster Session 10: Yeast Prions and Heat Shock Proteins

PS10-1: QN-Rich fragment of Gln3, but not full-length protein, forms Amyloid-Like aggregates in *Saccharomyces cerevisiae*

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Gln3 is a yeast transcriptional regulator of nitrogen metabolism harboring QN-rich region (Gln3QN), which is the key feature of all known amyloid-based yeast prions. Recently, this region was shown to form detergent-resistant amyloid-like aggregates. In our work, Gln3QN also formed dot-like aggregates, unlike full-length Gln3, which being fused with YFP showed diffuse fluorescence. Interestingly, the aggregate formation of Gln3QN significantly increases in the presence of [PIN⁺] – prion form of QN-rich protein Rnq1. [PIN⁺] is known to enhance *de novo* appearance of another one yeast prion – [PSI⁺], formed by Sup35, which also contains QN-rich region. Interestingly, [PSI⁺] not only does not induce Gln3QN aggregation, but even decreases the rate of Gln3QN aggregates in the presence of [PIN⁺]. In addition, it should be noted that aggregates of Gln3QN fused with YFP colocalize with high frequency with Rnq1-CFP aggregates in [PIN⁺] strain, which suggests that Rnq1-CFP aggregates may serve as templates for Gln3QN polymerization via interaction between their QN-rich regions. Considering the full-length Gln3 does not aggregate in the presence or absence of [PIN⁺] and [PSI⁺], we may propose that QN-rich region in full-length protein is buried inside the molecule that disturbs its aggregation propensity. Overall, the data obtained are important for understanding of mechanisms of interaction, aggregation and cross-seeding of QN-rich sequences. The study was supported by the Russian Foundation of Basic Research (14-04-32213), by the grant of the President of the Russian Federation (MK-4854.2015.4) and by the grant of St. Petersburg State University (0.37.696.2013). The authors acknowledge St. Petersburg State University for opportunity to use facilities of the Research Resource Center for Molecular and Cell Technologies.

PS10-2: Substitutions of polar amino acids by charged ones change Sup35NMp aggregates morphology

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In yeast *Saccharomyces cerevisiae* protein Sup35 is a release factor. It plays important role in translation termination. This protein is also essential for [PSI⁺] prion propagation. In prion conformation Sup35p forms amyloid aggregates and induces conversion of the cellular protein into its prion isoform. We previously described effects of *sup35^{KK}* mutations (designated M1 – M5) on [PSI⁺] properties: two alleles eliminate the prion, other change its properties (Bondarev et al., 2013). All studied mutations led to substitutions of the pairs of polar amino acids (QQ or QN) by charged amino acids (K) in one of oligopeptide repeats of N-domain Sup35p. The mutations were designed based on the model of super-pleated β -structure (Kajava et al., 2004) and was assumed to change structure of Sup35p aggregates. To prove this hypothesis we investigated properties of Sup35 proteins with corresponding substitutions (Sup35NM-MXp) *in vitro*. All of them spontaneously form SDS-resistant fibrillar aggregates with increased width compared to wild type (WT) protein (according to transmission electron microscopy (TEM)). This result supports expected structural changes. Addition of the sonicated fibrils to monomeric protein significantly decreases time of its aggregation because in this case protein molecules interact rather with preexisting aggregates and template their structure than spontaneously form new nuclei of aggregation. Next we used fibrils of Sup35NM-MXp to induce aggregation of native Sup35NMp. Further TEM analysis revealed that in all cases, except Sup35NM-M2p, width of obtained fibrils was increased compared to WT. Atomic force microscopy measurements also support observed changes in aggregates morphology, but not for all cases. In summary, these data prove that investigated mutations irreversible alter the structure of Sup35p aggregates and that modified conformations are templated by the WT protein. The authors acknowledge Saint-Petersburg State University for following research grants: 1.37.291.2015, 11.37.290.2015, 0.37.696.2013, 1.50.1041.2014, 1.41.528.2015, 15.61.2218.2013. This work was also supported by RFBR (13-04-00645).

PS10-3: Structure-based view on [PSI^+] prion properties

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Yeast [PSI^+] prion is one of the most well characterized system for the investigation of the prion phenomenon. However, until recently, the lack of data on the 3D arrangement of Sup35p prion fibrils hindered progress in this area. The recent arrival in this field of new experimental techniques led to the parallel and in-register superpleated β -structure as a consensus model for Sup35p fibrils. Recently we analyzed the effect of amino acid substitutions of the Sup35 protein through the prism of this structural model. The core structural element of a majority of naturally-occurring and disease-related amyloid fibrils is a β -arcade representing a parallel and in register stacks of β -strand-loop- β -strand motifs called β -arches. Based on an assumption that protein sequences that are able to form β -arcades are amyloidogenic, a computational program “ArchCandy” to predict amyloidogenic regions in proteins has been developed [1]. All PNM ($[\text{PSI}^+]$ -no-more) and antisuppressor mutations of Sup35p revealed by the spontaneous mutation screens [2-4] decrease the amyloidogenic potential predicted by ArchCandy. The observed destabilization of [PSI^+] prion in the proline-containing mutant alleles [5] can be explained by the decrease of the amyloidogenic potential predicted by ArchCandy. Also this tool was able to predict the increase of prion formation, related to the insertions of hydrophobic residues, within the first 25 residues of N-domain and [PSI^+] prion destabilization after deletion of tyrosines [6]. Finally we predicted effects of sup35^{KK} mutant alleles published in our previous work [7]. In agreement with the experimental data, ArchCandy assigns a lower amyloidogenicity score to alleles leading to prion loss (Y46K/Q47K and Q61K/Q62K) and predicts almost no effect on the fibril-forming potential for the other downstream sup35^{KK} alleles. Among sup35^{KK} , Q80K/Q81K leads to the strongest [PSI^+] phenotype. To explain this data, we proposed that these mutations makes the prion-forming region shorter and this increases the strength of [PSI^+] prion. Such correleation for other prion variants was already described in litterature. The authors acknowledge Saint-Petersburg State University for following research grants: 1.37.291.2015, 0.37.696.2013, 1.50.1041.2014, 1.50.2218.2013. This work was also supported by RFBR (13-04-00645, 14-04-32213).

[1] Ahmed et al., (2014) *Alzheimers & Dementia* 11, 681-690; [2] Doel et al. (1994) *Genetics*. 1994 Jul;137(3):659-70; [3] DePace et al. (1998) *Cell*. 93, 1241-52; [4] King (2001) *J Mol Biol*. 307, 1247-60; [5] Chang et al., (2008) *Proc Natl Acad Sci U S A* 105, 13345-50; [6] Gonzalez Nelson et al. (2014) *PLoS One*. 9, e89286; [7] Bondarev et al. (2013) *J Biol Chem*. 288, 28503-13

PS10-4: Formation of a Metastable Prion by the Yeast Actin Associated Protein Lsb2

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Amyloid formation in vivo is thought to result from alterations in protein homeostasis and cellular quality control system, however specific mechanisms remain elusive. We have shown that paralogous actin associated proteins Lsb1 and Lsb2 modulate maintenance of the [PSI^+] prion during thermal stress and that Lsb2 levels and Lsb1 processing are induced by heat-shock. Here we demonstrate that Lsb2 forms prion state [LSB^+]. [LSB^+] is transmitted through mating and meiosis, and is cured by guanidine-HCl. Detergent resistant Lsb2 aggregates, are detected in the cultures overproducing Lsb2 and are maintained by the [LSB^+] cells. [LSB^+] is mitotically unstable, and is lost by a significant fraction of cells during growth. In agreement that Lsb2 is a short-leaved protein degraded via ubiquitin-proteasome system, mitotic stability of [LSB^+] is increased in the cells defective in Lsb2 ubiquitination. Lsb2 derivative, deficient in association with the actin cytoskeleton, is unable to form detergent-resistant aggregates, convert into the [LSB^+] prion and promote conversion of Sup35 into [PSI^+] prion. Substitution of the Lsb2 8Q stretch to 8N decreases the average size of Lsb2 polymers and increases efficiency of [PSI^+] prion induction by overexpression of Lsb2 and Sup35. Lsb2 paralog, Lsb1 cannot induce [PSI^+], and this difference in prion-inducing abilities between two proteins can be traced to a single amino acid substitution. Our findings directly implicate the role of ubiquitin-proteasome system and actin cytoskeleton in formation of

metastable transient prions influencing amyloid formation by other proteins and shed new light on protein-based inheritance mechanisms of protein assembly diseases.

PS10-5: Analysis of interactions and prion transmission between yeast proteins with different homology levels

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Prions are self-perpetuating aggregated proteins associated with fatal diseases in mammals and controlling heritable traits in yeast. Transmission of mammalian prions between different species is usually impaired, due differences in the primary structures of prion-forming proteins. However, this barrier could be overcome, for example in case of ‘mad cow’ disease transmission to humans. Interspecies transmission barriers were also shown for yeast prions. We used a yeast Sup35/[*PSI*⁺] experimental system to explore prion transmission barriers, and studied Sup35 proteins from four yeast species that show from 90 to 60% of amino acid similarity in their NM regions including prion domains, namely *Saccharomyces cerevisiae*, *S. paradoxus*, *S. bayanus* and *Lachancea kluyveri*. In contrast to previous work where specific prion isolates were tested, we induced prions by overproducing a divergent protein, that produces multiple prion variants. Only the most closely related Sup35NM region from *S. paradoxus* (90% identity) could effectively induce [*PSI*⁺] in the *S. cerevisiae* cells. Fluorescence microscopy analysis confirmed previous data showing that *S. cerevisiae* protein coaggregates with the *S. paradoxus* or *S. bayanus* proteins in the *S. cerevisiae* cells, however FRET analysis demonstrated significantly lower efficiency of physical interaction between *S. cerevisiae* and *S. bayanus* proteins, compared to the *S. cerevisiae* and *S. paradoxus* combination. The most distantly related Sup35NM regions of *S. cerevisiae* and *L. kluyveri* showed neither coaggregation nor direct interaction. By using a newly developed computational approach, named ArchCandy, we have composed the spectra of prion structures generated by divergent prion domains. It turned out that this approach can accurately predict effects of the species barrier feature and cons impact of some amino acid substitutions on the species barrier. Further experimental analysis of species barrier predictions is currently underway. This work was supported by the St. Petersburg State University grants 1.37.291.2015, 1.50.1038.2014 and by RFBR grant 15-04-06650.

PS10-6: The longevity associated protein Sir2 modulates prion segregation in cell divisions after stress

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Budding yeast *Saccharomyces cerevisiae* practice asymmetric cell division, a process during which oxidized and other damaged proteins are preferentially retained in the mother cell while the daughter cell receives undamaged proteins. While the mechanisms of asymmetric cell division are not fully understood, it is known that this process is both required for the daughter cell to have full replicative ability and linked to cellular aging. The yeast protein Sirtuin 2 (Sir2) is a NAD⁺ dependent deacetylase that deacetylates histones, CCT chaperonin and other targets, and is crucial for asymmetric cell division and aging. Cells lacking Sir2 do not show mother cell specific accumulation of oxidatively damaged proteins after cell division and exhibit dramatically reduced replicative life spans. Here we show that the self-perpetuating aggregated (prion) form of the yeast protein Sup35 co-localizes with GFP-tagged protein Hsp104 after heat shock in a similar manner as previously shown for oxidatively damaged proteins. We also demonstrate that deletion of *SIR2* drastically decreases loss of Sup35 prion after heat shock, which has been linked to asymmetric segregation in our previous studies, and delays (but does not abolish) prion curing by overexpression of Hsp104 in non-stressed cells. Notably, Hsp levels are not altered in the absence of Sir2. Deletion of the gene coding for another sirtuin, Hst2, which is not implicated in asymmetric cell division, has only a mild effect on prion loss. Our data show that after stress, at least some yeast prions are controlled by the cell asymmetry machinery in the same way as aggregates of oxidatively damaged proteins.

PS10-7: Proteasome storage granules and misassembled proteasome aggregates are distinct proteasome inclusions

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Cellular toxicity introduced by protein misfolding threatens cell fitness and viability. Failure to eliminate these polypeptides is associated with various aggregation diseases. 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 misassembled proteasomal subunits are poorly understood. We reveal how the dysfunctional proteasome is controlled by the PQC machinery. We found that among the multilayered quality control mechanisms, UPS mediated degradation of its own misassembled subunits is the favored pathway. We also demonstrated that the Hsp42 chaperone mediates an alternative pathway, the accumulation of these subunits in cytoprotective compartments, and also distinguishes them from proteasome storage granules, proteasome aggregates that are formed upon carbon depletion. Thus, we show that proteasome homeostasis is controlled through probing the level of proteasome assembly, and the interplay between UPS mediated degradation or their sorting into distinct cellular compartments.

PS10-8: *SFP1* as an effector of prion-dependent lethality in yeast

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Studies of translation termination in yeast *Saccharomyces cerevisiae* are intertwined with studies of prions since at least three yeast prions ($[PSI^+]$, $[ISP^+]$, and $[NSF^+]$) are known to affect translational fidelity. $[PSI^+]$ and $[ISP^+]$, the respective prion forms of the release factor Sup35 and transcriptional regulator Sfp1, have antagonistic effects, *i.e.* suppression and antisuppression of nonsense mutations. Previously, we proposed a synthetic lethality test for genes that may influence properties of the translation termination factors Sup35 and Sup45. It is based on the fact that combination of most *sup45* mutations with $[PSI^+]$ prion in diploids is fatal. During studies of Q/N-rich transcription factors we found that additional expression of *SFP1* gene enhances the synthetic lethality by strengthening the $[PSI^+]$ phenotype, even though antisuppressor properties have been described previously for Sfp1 overexpression. Elevated expression of *SFP1* influenced both *SUP35* and *SUP45* mRNA levels, but we observed changes only in Sup35 protein level. Still we found that alteration of a putative Sfp1 binding site in the promoter of *SUP45* affects strain phenotype although very slightly. We conclude that, apart from its role in $[ISP^+]$ formation, Sfp1 might affect nonsense suppression via regulation of transcription of both *SUP35* and *SUP45*. The research was supported by RRC MCT SPbSU. The authors acknowledge Saint-Petersburg State University for research grants 1.37.291.2015, 0.37.696.2013 and Russian Foundation of Basic Research for research grants 13-04-00645 and 14-04-31265.

PS10-9: The importance of *S. cerevisiae* Hsp31p conserved Cys138 residue for the stability and subcellular localization of this protein

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S. cerevisiae Hsp31p belongs to the ubiquitous DJ-1/ThiJ/PfpI superfamily of proteins. On the basis of the crystal structures determined for a number of members of this family from various organisms, including Hsp31p and human Parkinson's disease-associated DJ-1, they share many structural features, yet they do not necessarily have similar molecular function(s). One of those features is single cysteine residue residing in a cavity of the molecule and forming, together with nearby histidine and glutamic acid, the so-called catalytic triad, found in many hydrolases and transferases. The Hsp31p catalytic triad most closely resembles those found in cysteine

proteases, despite the fact that recessed configuration of cysteine residue makes this enzymatic activity unlikely. We have previously shown the involvement of Hsp31p in the cell protection against oxidative stress. More recently the significance of this protein for stationary phase survival was demonstrated and the glyoxalase activity was ascribed to *S. cerevisiae* Hsp31p and to its homologs from two other yeast species. Nevertheless, the exact cellular role of this protein is still obscure. To gather more insight into the function of Hsp31p we studied the importance of its Cys138 residue. We have found that the protein devoid of this residue is less abundant than the wild-type protein in yeast cells exposed to stress conditions, but on the other hand it is resistant to oxidative stress-induced degradation. We postulate that the presence of Cys138 residue in Hsp31p polypeptide and its redox state determines Hsp31p stability. Funding: Polish National Science Center grant no.: 2011/01/B/NZ3/02904

PS10-10: Proteomic screenings for novel amyloid-forming proteins in yeast *Saccharomyces cerevisiae*

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Amyloids are protein fibrils with cross-beta structure. Recently, we developed a method for proteomic screening of amyloid-forming proteins called PSIA [1], which consists of three major steps: (i) purification of detergent-resistant protein fractions rich in amyloids, (ii) separation of proteins either by two dimensional gel electrophoresis or by high performance liquid chromatography followed by (iii) mass-spectrometric identification of proteins. PSIA was efficient for detection of different known yeast (Sup35, Rnq1, Bgl2) and mammalian (PrP, A β) amyloids. In addition, it allowed detecting of several yeast proteins, which probably form amyloid polymers *in vivo* at physiological conditions: Gas1, Ape1 and Ape4. We demonstrated that both, Ape1 and Gas1, fused with GFP form fluorescent foci when overproduced. Moreover, Gas1-GFP forms such foci at the physiological level of expression. Also, polymers of Ape1-GFP and Gas1-GFP are detected by semi-denaturing gel electrophoresis (SDD-AGE). Using PSIA we identified the proteins that determine the maintenance and manifestation of prion factor [*NS1* $^+$] that causes GuHCl-curable nonsense suppression in yeast strains with specific genetic background [2,3]. [*NS1* $^+$] strain, in contrast to [*ns1* $^-$], contains detergent-resistant polymers of two prion proteins, Swi1 ([*SWI* $^+$]) and Rnq1 ([*PIN* $^+$]). Also, presence of these two prions in the [*NS1* $^+$] strain causes amyloid-like aggregation of the key regulator of pseudohyphal growth, Mit1. Both, [*SWI* $^+$] and [*PIN* $^+$], are responsible for the nonsense suppression in the [*NS1* $^+$] strain: elimination of [*PIN* $^+$] significantly decreases nonsense suppression, while elimination of [*SWI* $^+$] results in the complete loss of the suppressor phenotype. Taking together, [*NS1* $^+$] represents a novel type of epigenetic factors, whose maintenance and manifestation depends on direct or indirect interactions between several prions. The study was supported by the grant of the President of the Russian Federation (Project MK-4854.2015.4). The authors acknowledge St. Petersburg State University for opportunity to use facilities of the Research Resource Center for Molecular and Cell Technologies. [1] Nizhnikov et al. (2014) *PLOS One* e116003; [2] Saifitdinova et al. (2010) *Curr Genet.* 56, 467-7; [3] Nizhnikov et al. (2012) *Curr Genet.* 58, 35-47.

PS10-11: Modulation of polyglutamine toxicity in yeast

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Expansion of the polyglutamine (polyQ) stretch in the human huntingtin protein leads to its aggregation and Huntington's disease (HD). Critical characteristics of HD can be modeled in yeast *Saccharomyces cerevisiae*. Constructs containing only expanded polyQ stretch of the huntingtin exon 1 fused to GFP, form toxic aggregates in yeast cells bearing endogenous QN-rich proteins in the aggregated (prion) form. Presence of the proline (P)-rich region targets polyQs to the large intracellular deposit, similar to mammalian aggresome (Wang et al. 2009 FASEB J. 23: 451). This ameliorates polyQ toxicity in cells containing the prion form of Rnq1 protein, but not in the cells containing the prion form of translation termination factor Sup35 (eRF3), where components of translation termination machinery are sequestered by polyQs (Gong et al. 2012 PLoS Genet. 8: e1002634). Thus,

one and the same mode of polyQ aggregation could be cytoprotective or cytotoxic, depending on the composition of other aggregates in a eukaryotic cell. To further investigate mechanisms controlling polyQ cytotoxicity, we analyzed an impact of the length of P-rich region on cytotoxicity, and screened for proteins whose absence antagonizes the cytoprotective effects of polyQP “aggresome”. Results of these screens will be discussed. This work was supported in part by the Russian Science Foundation grant 14-50-00069. The authors acknowledge the SPbSU Resource Centers “CHROMAS”, and “Molecular and Cell Technologies” for technical support.

PS10-12: Multimeric Ade2 protein attached to the prionogenic domain of the Sup35 protein induces appearance of the aggregates in both [PIN+] and [pin-] strains

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Prionization of the translation termination factor eRF3 (Sup35p) in the yeast *Saccharomyces cerevisiae* leads to the impairment of translation termination, which manifests phenotypically as nonsense suppression. Prionization of the Sup35 protein is accompanied with the appearance of amyloid fibrils, the structural core of which is N-terminal domain of Sup35p. The presence of the [PIN+] factor, which is a prion form of the Rnq1 protein, is usually necessary for induction of Sup35p prionization. C domain of the Sup35 protein is functionally active as translation termination factor. M domain serves as a linker domain. Chimeric proteins consisting of the N domain of Sup35 protein fused with amyloidogenic proteins from different organisms are prionized with high efficiency when these chimeric proteins are overproduced. Another way to increase prionizing properties of Sup35N may consist in the use of multimeric proteins attached to Sup35N. In our laboratory we have obtained chimeric proteins in which Ade2 protein of *S. cerevisiae*, multimeric enzyme acting on the adenine biosynthesis pathway, is attached to the Sup35N or Sup35NM domains. We showed that expression of the chimeric genes SUP35N-ADE2 and SUP35NM-ADE2 under the control of SUP35 promoter in [PIN+] strains leads to the prion conversion of chimeric protein and induces the prionization of the full-length Sup35p. The NM-Ade2 chimeric protein acts as more effective prion inducer than N-Ade2. In [pin-] strains we didn't see neither nonsense-suppression nor prion aggregates of N-Ade2 and NM-Ade2 chimeric proteins. In contrast, when we fused N-Ade2 and NM-Ade2 with GFP, we saw aggregates of the chimeric proteins not only in [PIN+] but in [pin-] strain too. Thus, the fusion of the prionogenic domain with the multimeric domain leads to the increase of non-prion aggregation, and the presence of the interjacent M-domain, which divides the prionogenic and multimeric parts, increases the efficiency of the prion conversion. Supported with St-Petersburg University research grants 0.37.696.2013 and 1.37.291.2015, RFBR grant 15-04-08159.

PS10-13: Search for new prions in yeast

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Amyloids are self-aggregating cross-beta fibers. Amyloid formation is associated with a variety of diseases in human and animals, including Alzheimer's and Parkinson's diseases, and type II diabetes. Transmissible amyloids (prions) could be infectious (in mammals) or heritable (in yeast). Recent evidence suggests that many proteins can form amyloid-like fibers, maintained permanently or transiently during a specific period of a protein “lifespan”. However, biochemical procedures specifically identifying amyloids formed by previously unknown proteins in the in vivo samples are lacking thus far. Yeast *Saccharomyces cerevisiae* contains a variety of amyloid-based prions and is frequently used for amyloid studies. We employ yeast for the development of approaches based at amyloid identification by genetic and biochemical tools. By using genetic approaches, we have identified a new prion, [MCS+]. [MCS⁺] causes a phenotype similar to the previously described prion [PSI⁺] (a prion form of the translation termination factor Sup35), but is not related to the Sup35 protein and can be phenotypically detected only in the absence of the Sup35 prion domain. We are also applying various biochemical approaches to identification of proteins responsible for [MCS⁺] and some other prion-like phenotypes. These approaches are based on detergent resistance, as well as on centrifugation and electrophoretic properties of amyloid aggregates, including a newly developed method of “agarose trapping”. If proven to work for yeast, these approaches can be adapted for identifying amyloids in different organisms including humans.

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Poster Session 11: Medically relevant yeasts and host microbe interactions**PS11-1: Investigating novel factors underlying oxidative stress resistance in the pathogenic yeast *Candida glabrata***

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For pathogenic yeast species, adaptation to stresses encountered in the human host is vital for survival and the establishment of infection. The ability of such species to mount a robust response to reactive oxidative species (ROS) encountered in the phagosome as part of the oxidative burst elicited by immune cells is imperative for survival following phagocytosis. The pathogenic yeast *Candida glabrata* is intrinsically more resistant to ROS than its close relative *Saccharomyces cerevisiae*, despite a high degree of similarity in the core oxidative stress responses between the two species. To characterise and elucidate novel factors contributing to oxidative stress resistance in *C. glabrata*, mutants resistant to oxidative stress-inducing chemicals hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (tBOOH) were generated using EMS mutagenesis and microevolution methods. Whole genome sequencing of the resultant 108 stress resistant strains revealed genome-wide polymorphisms and aneuploidy events. Recreation of selected polymorphisms in a *C. glabrata* background will verify the role of such mutations in oxidative stress resistance in this species. The fitness impact of acquiring stress resistance and the effect this may have on virulence was explored. The majority of oxidative stress resistant mutants were found to be more susceptible to another type of stress. Indeed, resistance to one type of oxidative stress did not confer resistance to other oxidative stress agents. Most strikingly, 70 % of these mutants were more susceptible to fluconazole, a major antifungal used for the treatment of *Candida* infections, and many show a fitness defect under non-stressed conditions. Additional competition experiments revealed that stress resistant strains tend to have a competitive fitness decrease. The effect of this observed stress resistance and fitness trade-off has on virulence is being investigated in a *Galleria mellonella* model of infection.

PS11-2: Genetic identification of the systems for active transport of riboflavin into the cell (permease) and out of cell (excretase) in the flavinogenic yeast *Meyerozyma (Pichia) guilliermondii*

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Riboflavin is a water-soluble vitamin (vitamin B₂) required for synthesis of the flavin coenzymes, flavin mononucleotide and flavin adenine dinucleotide. Cells of wild-type strains of the yeast *Meyerozyma guilliermondii*, cannot uptake riboflavin from the medium but are able to overproduce and excrete this vitamin under certain conditions. Previously two *M. guilliermondii* mutants able to active riboflavin transport were selected and shown to possess two distinct systems for riboflavin uptake. It was postulated that these systems are cryptic in wild-type strains. Several genes encoding putative riboflavin permeases were identified in *M. guilliermondii* genome by searching for homology to *Saccharomyces cerevisiae* riboflavin transporter Mch5p. Deletion of identified genes PGUG_04452 and PGUG_01089.1 in *M. guilliermondii* strain R93 that actively transported and accumulated riboflavin resulted in 3.5-4 and 18-20 fold decrease of riboflavin permease activity, correspondingly. In addition an insertion mutant IS2-2 which did not transport riboflavin into the cell at all was selected and was shown to be defective in gene PGUG_01642 that encodes a putative transporter belonging to MFS family. Deletion of this gene in *M. guilliermondii* strain R93 completely blocked riboflavin accumulation. Two *M. guilliermondii* genes PGUG_04776.1 and PGUG_05894.1 encoding transporters homologous to mammalian protein BCRP (breast cancer resistance protein which is involved in riboflavin extrusion into milk) were identified, cloned and deleted. Deletion of these genes did not affect phenotype of *M. guilliermondii* riboflavin accumulating strain R93. Introducing of the cloned native gene PGUG_04776.1 into cells of R93 did not alter its phenotype regarding the energy dependent riboflavin excretase activity. In contrast, most of selected

transformants bearing an additional copy of gene PGUG_05894.1 possessed 2-2.5 folds increased energy-dependent riboflavin excretase activity as compared to the recipient strain. Moreover about 30% of them possessed approximately 6 folds increase in activity of riboflavin excretase activity. Obtained results suggested that gene PGUG_05894.1 encodes a transporter involved in excretion of riboflavin by *M. guilliermondii*. Role of the identified transporters in the wild-type strains of *M. guilliermondii* will be discussed.

PS11-3: Characterization of cell wall enzymes expression profiles in *Candida glabrata* treated with echinocandins or polyenes

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Candida albicans and *Candida glabrata* are commensal fungi found on the microbial flora of the mucosal surfaces of the human body, though these can be opportunistic pathogens, especially in immuno-compromised individuals. *C. albicans* and *C. glabrata* constitute 65-75% of invasive candidiasis. *C. glabrata* is associated with higher patient mortality and is emerging as an important nosocomial non-*Candida albicans* species due to its increasing drug resistance, often succeeding other *Candida* fungi in patients undergoing long-term antibiotic and antifungal therapy. *C. glabrata* has been shown to be resistant to echinocandins, an important group of antifungal drugs known to inhibit synthesis of the major fungal cell wall polysaccharide beta-(1,3)-glucan, but is susceptible to polyenes such as amphotericin B. The *C. glabrata* cell wall is an important virulence factor for host invasion, stress resistance and immune evasion. The fungal cell wall mainly consists of polysaccharides and in *C. glabrata* it is made of an insoluble network of glucan and chitin fibrillar linkages. Two key enzymes that synthesise these polymer components are plasma transmembrane 1,3-β-D-glucan synthase and chitin synthase III. In our report, we characterized the effects of the drugs on the growth and viability of *C. glabrata* treated with either caspofungin or amphotericin B. We also present data on the levels of cell wall enzyme transcripts of *C. glabrata* exposed to such treatments using Real-time PCR. To correlate changes in the transcript levels of these enzymes and cell wall integrity, we also performed microscopic examination of cell wall stainings of treated 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.

PS11-4: Population genomics of *Saccharomyces cerevisiae* human isolates reveals adaptation to the gastrointestinal tract

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Despite in-depth knowledge of the genetic, molecular and phenotypic traits regulating the physiology of *Saccharomyces cerevisiae*, the forces shaping its origin and evolution are still debated. *S. cerevisiae* has been associated to human activities so deeply to harbour the notion of being a domesticated organism. The quest for the ecological niches of *S. cerevisiae* has led to examine its population structure, and to classify with respect to the source and the type of human activity from which it derived. Since human exposure to fungi is constant, recent studies have begun to note that the mycobiota, the commensal fungal community, is a significant player in

host-microbe interactions. A recent hypothesis is that human environment-associated *S. cerevisiae* give rise to clinical strains causing colonization/infection. A few studies investigated fungal communities in chronic inflammation, especially in Inflammatory Bowel Diseases (IBD), and the production of anti-*Saccharomyces cerevisiae* antibodies (ASCA), one of the diagnostic markers of Crohn's disease (CD). Here we present the genetic structure of a previously unknown populations of yeasts associated with human gut and especially with pediatric CD patients. *S. cerevisiae* strains isolated from the human gut showed clonal expansion and a unique cell wall composition with increased galactose and decreased mannose, thus suggesting selection and adaptation to the gut environment. A systems level approach, combining whole genome sequencing with immune-phenotyping of gut isolates, discovered selection on genes involved in sporulation and cell wall remodeling as crucial for the evolution of *S. cerevisiae* in the gut. Classifying gut strains according to their immunomodulatory properties, we discovered a set of genetically homogeneous isolates capable of inducing anti-inflammatory signals via regulatory T cell proliferation and another group of isolates with a mosaic genome, eliciting inflammatory immune response. Sporulation is associated with strain-specific differences in the cytokine pattern and with ASCA marker in CD patients, thus reflecting the yeast's ability to induce different inflammatory responses. We provide evidence that cell wall remodeling and sporulation ability is crucial for live in the gut and therefore we propose the role of the human gut in shaping *S. cerevisiae* evolution.

PS11-5: Gain-of-function overexpression screens to identify genes important for *C. glabrata* stress adaptation

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C. glabrata is an opportunistic pathogen that has contributed to the noticeable rise in fungal infections related to non-albicans *Candida* species in recent years. Since its designation as a pathogen by Wickham in 1957, relatively little is known about its mechanism of virulence. Phylogenetically, *C. glabrata* is more closely related to the non-pathogenic model organism *S. cerevisiae* than to other *Candida* species and approximately 77% of *C. glabrata* proteins have orthologues in *S. cerevisiae*. As adapting to the host environment is essential to its ability to infect the host, *C. glabrata* is well adapted to coping with environmental stress both within and outside of the host. In particular, *C. glabrata* has been shown to be much more resistant to growth under conditions of high osmotic and oxidative stress compared to *S. cerevisiae*. To begin to understand why *C. glabrata* is a pathogen yet its close relative *S. cerevisiae* is not, we carried out screens to identify *C. glabrata* genes important for adaptation to environmental stress. We constructed a partial *C. glabrata* ORFeome using Gateway® Technology consisting of approximately 2688 ORFs and transferred them into the Gateway® destination vector, pAG424GPD-ccdB, to form a pooled *C. glabrata* library. The pooled *C. glabrata* library was transformed in *S. cerevisiae* CG1945 and gain-of-function overexpression screens were carried out to identify *C. glabrata* genes that enabled *S. cerevisiae* to survive lethal stress conditions. As expected, analysis of the *C. glabrata* genes identified in the screens revealed many genes involved in the stress response. However a proportion of the genes identified are unique to *C. glabrata* suggesting that uncharacterised *C. glabrata* genes play an important role in its ability to survive stress and perhaps its ability to be a successful pathogen. Further characterisation of these genes will help us to understand *C. glabrata*'s mechanism of virulence.

PS11-6: RNAi as a tool to study virulence in the pathogenic yeast *Candida glabrata*

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Candida glabrata is one of the main pathogens causing mucosal and systemic infections in human. Systemic infections caused by this yeast have high mortality rates and are difficult to treat due to its intrinsic and

frequently further adapted antifungal resistance. To understand and treat *C. glabrata* infections, it is essential to investigate the molecular basis of *C. glabrata* virulence and resistance. However, *C. glabrata* virulence is not well studied and gene deletion protocols are time consuming and often inefficient and, furthermore, inappropriate for the disruption of essential genes. We have established an RNA interference (RNAi) protocol in *C. glabrata* by expressing Dicer and Argonaute genes from *Saccharomyces castellii*. Our results using reporter genes and putative virulence genes show that introduced RNAi results in 75–95% gene knockdown depending on the construct type (antisense or hairpin). The RNAi strain was further used as a basis for antisense gene library based on a multi-copy replicative plasmid. Transformants were subjected to phenotypic profiling using high-resolution quantification of growth in search of genes involved in cell integrity, antifungal drug and ROS resistance. For example, one of the amphotericin B sensitive transformant obtained was carrying an antisense plasmid for *C. glabrata* uncharacterized gene, CAGL0I00116g. The genes identified by this approach may prove to be new potential targets for the development of anti-*C. glabrata* therapies.

PS11-7: The haploid nature of *Candida glabrata* is advantageous under harsh conditions

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Candida glabrata is the second most prevalent yeast pathogen in humans. Systemic infections caused by this pathogenic yeast have high mortality rates and are difficult to treat because it readily develops resistance in response to drug exposure during treatment. In contrast to other human yeast pathogens and the closely related *Saccharomyces* yeasts, *C. glabrata* has only been found haploid and asexual yeast. We asked if its haploid nature and the observed genome rearrangements could be an advantage for *C. glabrata* to survive *in vivo*. To address this question, the competition between haploid and artificially created diploid strains of *C. glabrata* was studied *in vivo* (in a fly and a mouse model) and *in vitro* under normal and stress conditions (fluconazole, high temperature). Experimental populations (competition groups) of 2 haploid parental strains and one diploid (a fusion product of the corresponding parental haploids) were used in competition experiments, and the outcome was analyzed. We showed that after few days in most cases haploid strains outcompeted the diploid one in infected flies and mice. The haploid fraction increased but the diploid cells decreased in number *in vivo*. When this experiment was done competed *in vitro*, the diploid strains always prevailed under non-stressed conditions. However, with increasing fluconazole concentrations and at elevated temperatures the haploid strains outcompeted the diploid one more often. Thus, the haploid nature seems to provide an advantage in the competition under harsh conditions. Some of the prevailing strains were analyzed for their gene expression, showing that several genes drastically changed their expression.

PS11-8: Drug resistance and adhesion: a closer look at the Dark side of the wall

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Stress conditions and presence of antifungal drugs induce significant changes in the cell wall composition of yeasts and fungi. The molecular architecture of the cell wall is also modified in these conditions, particularly the nature, repartition and attachment of cell wall proteins to the cell surface. Atomic Force Microscopy (AFM) is a powerful tool for studying the morphology, nanomechanical and adhesive properties of live microorganisms

under physiological conditions. We took advantage of the most recent AFM technological developments to image and measure the biophysical consequences of these various stresses on *C. albicans* cell morphology at the nanoscale, focusing on changes in cell surface aspect and characteristics: roughness, elasticity, adhesion forces. We notably explored the effects of the antifungal drug caspofungin used in human health [1]. Our investigation revealed a deep cell wall remodeling induced by this drug, evidenced by a dramatic increase in chitin and decrease in beta-glucan content. Remarkably, a low dose of caspofungin (0.5 x MIC) resulted in characteristic expression of adhesins on *C. albicans* cell surface. Moreover, in order to get a better understanding of *C. albicans* adhesion mechanisms, we performed Single Molecule Force Spectroscopy (SMFS) experiments to visualize the adhesins organization and to quantify the adhesion forces. We were able to map the adhesins at the cell surface and to distinguish between hydrophobic and specific affinity interactions [2]. Combined with molecular biology tools, this approach also enabled us to unravel the particular contribution of previously uncharacterized proteins (PGA22 and PGA59) to *C. albicans* adhesion mechanism [3]. In the future we will focus on new approaches using Single Cell Force Spectroscopy with AFM and Optical Tweezers as well as Sheer-Stress Flow Chamber to study adhesion from the molecule scale to the population scale.

[1] Formosa C. et al., (2013) AAC 57, 3498; [2] Formosa C. et al.,(2015), Nanomedicine NBM 11, 57; [3] Cabral V. et al., (2014) PLoS Pathog 10, 1371.

PS11-9: Role of the Mycobiota in Multiple Sclerosis

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Multiple Sclerosis (MS) is an immune-mediated process in which an abnormal response of the body's immune system is directed against the Central Nervous System (CNS). To date the cause of MS is still unclear but it is possible that unidentified environmental factors could trigger the disease in predisposed individuals. The human gut is colonized by trillions of microorganisms that shape a unique ecosystem within different functions. Key roles of the microbiota are the modulation and the education of the host immune system; these mechanisms may be players in the development of multiple sclerosis. While metagenomics studies targeted at the bacterial component did not provide a significant difference within microbial component of the disease, the presence of a Th17 response suggested a role of the fungal component of the microbiota, the mycobiota, in the modulation of MS. Understanding the interaction of the mycobiota with the host might provide new insights into the pathogenesis of disease, as well as novel avenues for preventing and treating intestinal and systemic disorders. We characterized the gut mycobiota of 27 Multiple Sclerosis (MS) patients and 21 Healthy Subjects (HS) through culture-based analysis in order to understand the implication of intestinal fungi in onset of the disease. The analyses included also monozygotic twins to comprehend the influence of the genetic background on MS and microbiota. The isolated fungi showed a significantly increase in terms of abundance and richness in MS patients compared to healthy subject. We found also significant differences between diseased and healthy twins. We discovered the genus *Penicillium* more abundant in MS subjects than in HS. Amongst the isolates equally present in diseased and healthy twins, we observed the presence of *S.cerevisiae*, suggesting food borne fungi to be similarly important to those with an environmental origin.

PS11-10: A genome-wide transcriptional analysis of the response to hyphal wall stress in *Candida albicans*

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During the yeast to hypha (Y-H) transition, *Candida albicans* acquires attributes essential for adhesion and penetration into the tissues, two important processes for the establishment of invasive infections in immunocompromised patients. Cell wall °(1,3)-glucan remodeling catalyzed by Phr1p is required for hypha elongation, adhesion and virulence and Phr1p is a promising target for new antifungal agents. We exploited the pH-conditional nature of a *PHR1* null mutant to analyze the genome-wide transcriptional response to hyphal wall stress (HWS) during the Y-H transition. The changes include increase of transcript levels for eight mannoproteins, for the enzyme required for polymer cross-linking to chitin (*CRH11*), two chitin synthases (*CHS2* and *CHS8*), a chaperone of ER-export of Chs3p (*CHS7*) and reduction of adhesins, indicating adjustments in hyphal wall structure. Additionally, up-regulation of DNA replication and cell-cycle genes was associated with premature entry into S-phase. The *CCP1* transcript for the protein phosphatase Cek1p MAP kinase, constitutively hyperactivated by HWS, was more abundant in the mutant. Chitin level increased in the mutant and the deletion of *CHS3* was synthetically lethal with deletion of *PHR1* whereas *CHS2* and/or *CHS8* were dispensable. The *chs3 phr1* mutant showed a synthetic lethal phenotype on liquid or solid M199-pH 7.5 media. On Spider a physiological adaptation of the double mutant occurred at pH 7.5 whereas at pH 8 cells died. Therefore, HWS compensation is influenced by the filamentation conditions used and in less-demanding media adaptation occurs.

PS11-11: Commensal yeast *S. cerevisiae* trains human monocytes for a heightened cytokine response upon bacterial encounter

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The immune system is essential to maintain the mutualistic homeostatic interaction between the host and its micro- and mycobiota. Living as a commensal on human skin and being a passenger in the digestive tract, *Saccharomyces cerevisiae* could potentially modulate the host immunity and significantly shape the immune response. We observed that diverse *S. cerevisiae* strains induce trained immunity in monocytes through a strain-dependent manner leading to enhanced cytokine production upon secondary stimulation with TLR ligands and bacterial commensals. These features are reflected by the differences in the pro-inflammatory properties dependent on the origin of the strains, which may be potentially related to the different adaptation to the environment from which they were isolated. We established that even though β-glucan is sufficient to train the innate immunity, *S. cerevisiae* chitin drives the induction of trained immunity potentiating cytokine modulation and killing ability. This study reveals how commensal and passenger microorganisms could be important in promoting health and preventing mucosal diseases by modulating host defense and regulating the microbiota. Dietary supplementation of specific probiotic microorganisms may be a viable strategy to train a healthy immune system. This work was supported by funding from the European Community's Integrative Project FP7, SYBARIS (Grant Agreement 242220, www.sybaris.eu) and by funding from Provincia Autonoma di Trento's Accordo di Programma (METAFOODLABS project).

PS11-12: *C. albicans* with different genomic background reveal diverse host adaptation and differential processing by phagocytes

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Candida albicans is an important opportunistic yeast causing infections in susceptible host, but in healthy conditions is an harmless commensal. The pathogenicity of *C. albicans* is associated to either genomic or phenotypic characteristics that enable it to rapidly adapt to changing environmental, external signals, and help it in colonizing the host. The cell-hyphal transition is an example of virulence switching of *C. albicans* that promote tissue invasion and evasion of the host immune system. To investigate the intra-species variability in the phenotypical changes and immunoreactivity of *C. albicans*, we analyzed the whole genome sequences of 2 clinical strains (YL1 and YQ2). Whole genome analysis showed an intriguing genomic plasticity, an extreme variability and divergence between strains. Indeed, over the high polymorphism, strain-specific gene losses, acquisition, and several miss-sense genes were found. The most polymorphic genes codify proteins related to the cell wall and hyphal formation, suggesting a continuous adaptation to adverse environments or stress conditions. Genomic data were confirmed by phenotypical characterization showing changes in virulence related traits. Furthermore the fungal isolates were evaluated for their susceptibility and killing to microglia cells, and phagosome maturation in the BV2 microglia cells, used as an *in vitro* infection model. Although comparable in their susceptibility to phagocytosis by BV2 cells, these strains showed striking differences in term of intracellular survival. The YL1 isolate, in contrast to YQ2, resisted indeed to intracellular killing and eventually replicated inside the microglia. Moreover, we found a significantly lower percentage of YL1-containing acidic phagosomes, as compared to those observed in the YQ2-infected BV2 cells. These data suggest that YL1 may impair bactericidal activities of the microglia by inhibiting phagosome maturation. The increased virulence of YL1 shown in *in vitro* model appears to correlate with a different genetic makeup of this strain, particularly in genes involved in the pathogenesis of *C. albicans*. Our observations demonstrate that the nature and genomic features of *C. albicans* isolates dictate their adaptation to host environment generating phenotypic variability, which will translate into differential processing by phagocytes. Overall these results provide significant insights regarding the link between host adaptation, pathogenesis and evolution.

PS11-13: *Candida albicans* as a model in study on the mechanism of antifungal action of *Galleria mellonella* lysozyme

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Candida albicans is an opportunistic polymorphic pathogenic fungus, a common inhabitant of human gastrointestinal and reproductive tracts. Usually this commensal fungus is tolerated because the host immune system senses it as a low danger signal. However, as a consequence of local mucosal microenvironment disruption, it can rapidly proliferate and penetrate various physiological barriers. Depending on the potential of the host immune system, the response to such local changes could result in: complete elimination of the *Candida* cells, restoration of a previous state of mucosal commensalism, development of a chronic inflammatory response to mucosally localized *Candida*, and development of systemic disseminated candidiasis, when the immune system is not effective enough. In turn, in response to the host immune mechanisms, *C. albicans* undergoes morphological switch from yeast to more resistant pseudohyphal and/or hyphal forms, characteristic for established candidiasis. Lysozyme constitutes an important component of the humoral immune response against invading pathogens in animals. This protein is well known antimicrobial polypeptide exhibiting antibacterial and antifungal activities. Antibacterial action of lysozyme is related to enzymatic muramidase activity and to

non-enzymatic activity resembling a mode of action of cationic defense peptides. However, the mechanism of lysozyme fungistatic and/or fungicidal activity is not clear. Our previous study revealed that purified *Galleria mellonella* lysozyme, which like its human counterpart belongs to c-type family of lysozymes, bound to the cell surface of different filamentous fungi and yeasts including *C. albicans*. Moreover, *G. mellonella* lysozyme inhibited *C. albicans* growth at a relatively low concentration (0.5 µM). Our present research focuses on explaining the mechanism of anti-*Candida* activity of *G. mellonella* lysozyme. We proved the inability of the lysozyme to degrade standard chitinase substrates, which indicates that *G. mellonella* lysozyme could reduce the fungal growth through a non-enzymatic mode of action. Staining of lysozyme-treated *C. albicans* protoplasts with FITC-conjugated Annexin V and JC-1 dye showed that *G. mellonella* lysozyme can induce apoptosis in *C. albicans* cells. In addition, studies with the use of potassium channel inhibitor – tetraethylammonium chloride (TEAC) – revealed that *C. albicans* killing by the lysozyme is associated with ionic balance disruption. The work was supported by the Grant No 2013/11/N/NZ6/00535 (Decision No: UMO-2013/11/N/NZ6/00535) from National Science Centre (Kraków, Poland).

PS11-14: Biodiversity of the human gut mycobiota and its adaptation to the gastrointestinal tract

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The role of fungi as commensals has been neglected for long time and only recently few reports have explored the composition and dynamics of the human gut mycobiota. Commensal fungi are important in human health and disease and changes in commensal fungal populations have been shown to deeply affect pathologies not directly related to fungi, such as Inflammatory Bowel Diseases and Cystic Fibrosis. We studied the fungal gut populations of 111 healthy subjects using a culture-based approach characterizing the isolated fungi for commensalism-related traits. Fungi were detected in 80.2% of subjects leading to the identification of 349 different fungal isolates belonging either to *Ascomycetes*, *Basidiomycetes* and *Zygomycetes*. We found 34 different fungal species, some of which previously isolated solely in environmental samples, phenotypically adapted to be putative commensals of the human gastrointestinal tract. The 39.8% of inspected individuals has been found to carry at least one *C. albicans* isolate, resulting the most abundant and common yeast species found in ours samples. Analyses of fungal populations' dynamics suggest that the human gut mycobiota is relatively stable through the lifetime of individuals but significantly differ in a gender-related fashion.

Poster Session 12: Yeast sociobiology-sensing and signaling**PS12-1: An ATP analog sensitive version of Slt2 to study phosphorylation processes mediated by this MAPK****Esmeralda Alonso-Rodríguez, Pablo Fernandez Piñar, Humberto Martín, María Molina***Department of Microbiology II, University Complutense of Madrid, IRYCIS, Madrid, Spain*

MAPKs operating in signaling pathways catalyze the transfer of the \square -phosphate from ATP onto their substrates. In *Saccharomyces cerevisiae*, one of these routes is the cell wall integrity pathway (CWI), which mediates the response to cell wall stress. The MAPK of this pathway Slt2 phosphorylates the transcription factors SBF, composed of Swi4p and Swi6p, and Rlm1, which is responsible of the major transcriptional response. Additional known targets of Slt2 are the silencing protein Sir3 and components of this pathway such as the Rho1-GDP-GTP exchange factor Rom2, the MAPKKs Mkk1 and Mkk2, and the protein phosphatase Msg5, a negative regulator of Slt2. However, functional studies clearly suggest that there must be additional Slt2 substrates that remain to be identified. In order to have a new tool for gaining insight into Slt2 phosphorylation processes and to identify novel substrates of this MAPK, we have developed an ATP analog-sensitive version of Slt2 (*slt2-as*) in which the active site of this kinase is engineered to accept bulky ATP analogs. This version is functional but is inhibited by these ATP analogs. Analog-sensitive kinases also accommodate ATP analogs in which the \square -phosphate is replaced with a thiophosphate moiety, and thus these kinases are able to thiophosphorylate their substrates. Treatment of the thiophosphorylated proteins with a thiol-specific alkylating agent allows their easy detection through immunoblotting with a thiophosphate ester-specific antibody. Here we show that Slt2-as thiophosphorylates Rlm1 and Msg5 in *in vitro* kinase assays. Msg5 is phosphorylated both in the regulatory amino- and the catalytic carboxi-terminal domains. Furthermore, we have identified three novel Slt2 substrates, which are phosphorylated by this MAPK when produced as endogenous proteins but also when expressed as *E. coli* recombinant proteins. These substrates directly interact with Slt2 as revealed by *in vitro* copurification assays.

PS12-2: In silico functional analyses of adaptation to high ethanol**Ahmed Arslan, Vera van Noort, Kevin Verstrepen, Karin Voordeckers***KU Leuven, Belgium*

The process of molecular evolution can be recreated in lab settings to increase our knowledge about evolution. Experimental evolution has been studied in prokaryotes as well as unicellular eukaryotes like *S. cerevisiae*. We subjected this unique evolutionary model to study the adaptive evolution to high ethanol tolerance. During the course of the experiments the ethanol concentrations were gradually raised and extracted DNA was subjected to DNA sequencing to identify mutations. We computationally analysed these data to identify the mutations that have an impact on protein functional regions and interactions with other proteins. In silico, these mutations could lead to malfunctioning proteins and their underlying pathways.

PS12-3: Pterostilbene treatment impacts the flocculation behaviour in *S. cerevisiae* through transcriptional up regulation of F box encoding *AMN1* gene**Narendra Bairwa, Meenu Sharma***School of Biotechnology, Sri Mata Vaishno Devi University, Katra, Jammu & Kashmir, India*

Pterostilbene is a naturally occurring phenolic compound which exhibits anticancer properties. *S. cerevisiae* F-box encoding gene *AMN1* plays a key role in mitotic exit and cell separation. *AMN1* gene also has been implicated cell clumping behaviour besides the members of the FLO gene family. Here we have studied the expression profiling database (GEO) of *S. cerevisiae* for the expression status of the *AMN1* and its downstream genes involved in the regulation of the clumping behaviour namely *DSE1*, *DES2*, and *SCW11*. The expression status of the *ACE2* gene was also analysed which is transcriptional regulator of the *AMN1* gene. We observed the consistent transcriptional up-regulation of the *AMN1*, *ACE2* and downstream genes upon treatment with pterostilbene. Based on the analysis of the transcriptional data set we hypothesized that pterostilbene might influence flocculation behaviour of the *S. cerevisiae* cells through the up-regulation of the *AMN1* gene and its transcription regulator.

PS12-4:Characterization of PKA dependent phosphorylation sites on Ira2 RasGAP and their role in feedback regulation of the cAMP pathway in *Saccharomyces cerevisiae*

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In *S. cerevisiae*, cAMP/PKA pathway plays a major role in metabolism control, stress resistance and proliferation. PKA activity is regulated by cAMP level synthesized by adenylate cyclase, activated by Gpr1/Gpa2 GPCR system and Ras G proteins with their regulators, Cdc25/Sdc25 guanine exchange factors and Ira1/Ira2 GTPase activating proteins. Ira1 and Ira2 are homologs of human neurofibromin 1 (NF1) protein, whose mutations were found to be involved in human neurofibromatosis. The yeast RasGAP proteins share similar aminoacidic sequence and have related, but not identical, functions. Feedback regulation on RasGAP activity was previously proposed but it is still unclear at the molecular level. Pescini *et al.* (2012) determined in a quantitative way that only the presence of a feedback control on the activity of GAPs can induce the stable oscillatory regimes of cAMP: according with their computational analysis, Ira2 protein could be phosphorylated by PKA to downregulate the cAMP signal transduction pathway. Since online prediction tools suggested a PKA phosphorylation consensus sequence (RRNS) just near the catalytic site of Ira2, the serine 1745 was mutated in glutamate, to mimic a constitutive phosphorylated condition: the heat shock test performed to indirectly assay the activity of the cAMP/PKA pathway suggested that this aminoacid substitution reduced GAP activity, suggesting that S¹⁷⁴⁵ is not a target of the negative feedback control by PKA. PhosphoGRID, an online database of experimentally verified *in vivo* protein phosphorylation sites in yeast, suggests that the only other serine in the Ira2 sequence that is phosphorylated by PKA is the residue 1018 in the RRYR consensus sequence. Also the mutation of this serine to glutamate, generating the Ira2^{S1018E} mutant, should mimic a constitutive phosphorylated state. This non-conservative substitution has a poor effect on cell volume and resistance to heat stress, but the observed cell phenotypes rather suggest a slight increase of the pathway activity. Since PhosphoGRID doesn't indicate any other pKA-phosphorylation site on Ira2 sequence, we will further investigate if the phosphorylation on serine 1018 is a required but not sufficient event in the regulatory mechanism and if the RasGAP could be regulated by phosphorylation by other kinases.

PS12-5: The Msn2 mediated stress response: Survival based on “hedging your bet” and a dynamic interplay of transcription factor binding and nucleosome occupancy

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Yeast cell subjected to many different stresses elicit an acute transcriptional stress response mediated by the Msn2 transcription factor, which alters expression of both a stress specific-cohort of genes as well as a common cohort of genes that changes expression in a stereotypic fashion upon exposure to any of a wide variety of stresses. We have shown by dynamic single cell analysis that stresses regulate Msn2 activity through cytoplasm to nuclear relocalization but do so in an unusual way: stresses induce increased frequency of bursts of short-lived, recurrent periods of Msn2 nuclear localization with different stresses eliciting different patterns of bursts. Moreover, genetically identical cells subject to an identical stress can behave quite differently, with some cells mounting a robust nuclear occupancy of Msn2 while others show no nuclear localization at all. We have proposed that this idiosyncratic behavior allows populations of cells to “hedge their bet” as to what will be the optimum strategy for surviving the ensuing stress. We have used computational modeling and single cell analysis to determine that bursting is a consequence of noise in the stress signaling pathways amplified by the small number of Msn2 molecules in the cell. Moreover, we have applied genome wide chromatin immunoprecipitation and nucleosome profiling to address how different stresses determine where Msn2 binds under a particular stressful conditions, and thus what genes are regulated by that stress, and how that binding affects, and is affected by, nucleosome positioning and other transcription factor binding. These results provide *in vivo* validation of Widon’s model of indirect cooperativity of transcription factor binding, mediated by partial unwinding of nucleosomes by one transcription factor to allow access for a second transcription factor to a previously occluded binding site. Finally, we have addressed the “bet hedging” hypothesis by showing that

persistence of the Msn2-mediated stress response yields cell growth arrest and have identified the targets responsible for that growth arrest. We have applied experimental evolution paradigms to address the relative fitness of cells exhibiting stochastic stress responses versus those with a uniform response. In short, our results indicate that the stress response is complex and that complexity is critical for cell survival.

PS12-6: In *Saccharomyces cerevisiae* both sensing and metabolism of glucose regulate cell size

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Besides being the favorite carbon and energy source for *Saccharomyces cerevisiae*, glucose can act as a signaling molecule to regulate multiple aspects of yeast physiology. Addition of glucose to quiescent or ethanol growing cells triggers a fast and massive reconfiguration of the transcriptional program, which enables the switch to fermentative metabolism and promotes an outstanding increase of the cell biosynthetic capacity. Glucose signaling in yeast requires in most cases at least partial metabolism of the sugar: as a result, the roles of glucose as nutrient and signaling molecule are closely intertwined and it is difficult to separate the two functions. A central issue in this study was to determine whether (and possibly, to which extent) the regulatory function of glucose can be separated from its nutrient, fuel-supplying function. To this aim, we characterized (i) yeast strains in which glucose metabolism is strongly reduced or even prevented due to the absence of a functional transport system (hxt-null strain) or to the loss of the three kinases catalyzing the first step in glycolysis (hxa2 hxa1 glk1 strain); (ii) yeast strains defective in glucose sensing mechanisms due to the inactivation of extracellular glucose receptors encoding genes (GPCR-system (Gpr1/Gpa2 branch of the cAMP/PKA pathway) and Snf3/Rgt2 pathway). Our findings indicate that glucose may modulate yeast cell size by acting as a signaling molecule in a way partially independent from its role as nutrient. In fact, wild type yeasts exhibit a cell size modulation which is dependent on the extracellular glucose concentration and that is partially lost when the sugar sensing systems are inactivated. Furthermore, during an ethanol/glucose nutritional shift-up glucose induces a significant increase of cell size even in strains where sugar metabolism is completely abolished. However, in the absence of sugar metabolism, the glucose-dependent modulation of cell size is only transient and is substantially abolished following the loss of the sugar sensing pathways activities. In conclusion, the initial effect of glucose on yeast cell size during a nutritional shift-up may rely on sugar sensing and be partially independent of sugar metabolism; in contrast, long-term maintenance of “large size phenotype” would require glucose metabolism. These data provide a general framework that can be used to expand current models of yeast cell cycle and metabolism to include nutrient glucose sensing.

PS12-7: Nitrogen catabolite repression is sustained by signals distinct from glutamine and glutamate reservoirs

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Nitrogen Catabolite Repression (NCR) is a wide transcriptional regulation program enabling baker's yeast to downregulate genes involved in the utilization of poor nitrogen sources when preferred ones are available. Nowadays, glutamine and glutamate, the major nitrogen donors for biosyntheses, are assumed to be key metabolic signals regulating NCR. NCR is controlled by the conserved TORC1 complex, which integrates nitrogen signals among others to regulate cell growth. However, accumulating evidence indicate that the TORC1-mediated control of NCR is only partial, arguing for the existence of supplementary regulatory processes to be discovered. In this work, we developed a genetic screen to search for new players involved in NCR signaling. Our data reveal that the NADP-glutamate dehydrogenase activity of Gdh1 negatively regulates NCR-sensitive gene transcription. By determining the total, cytoplasmic and vacuolar pools of amino acids, we

show that there is no positive correlation between glutamine/glutamate reservoirs and the extent of NCR. While our data indicate that glutamine could serve as initial trigger of NCR, they show that it is not a sufficient signal to sustain repression and point to the existence of yet unknown signals. Providing additional evidence uncoupling TORC1 activity and NCR, our work revisits the dogmas underlying NCR regulation. This work is funded by the Commission Communautaire Française (COCOF) and by the Fonds de la Recherche Fondamentale Collective (FRFC 2.4547.11). MFK is FNRS Research Fellow (Fonds de la Recherche Scientifique). AMM is a senior research associate of FNRS.

PS12-8: Scaffolding activity of the MAPKK Pbs2p during the endoplasmic reticulum stress response in *Saccharomyces cerevisiae*

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Some environmental stimuli or changes in cellular processes may lead to deficiencies in protein processing and folding, which induce their accumulation and generate a condition known as endoplasmic reticulum stress (ERS). There are ER systems able to sense misfolding of newly synthesized proteins and therefore trigger a transcriptional program known as the unfolded protein response (UPR), needed to restore cellular homeostasis. In the yeast *Saccharomyces cerevisiae*, the high osmolarity glycerol (HOG) pathway, a MAPK transduction system that participates in the osmotic stress response, has been also involved in response to endoplasmic reticulum stress inducers. However, it appears that the role of the HOG components in the ERS requires different architecture and mechanism compared to their role in the high osmolarity response. For example, it has been seen that contrary to the hyperosmotic response, the Hog1p phosphorylation and the Pbs2p kinase activity may be dispensable to generate an adequate ERS response. In this work we propose that the scaffold domains of MAPKK Pbs2p are essential and sufficient to establish a response to the antibiotic tunicamycin, an N-glycosylation inhibitor, which generates ERS. For this, we generated deletions on the kinase and scaffold domains in Pbs2p, and we tested the ability of these constructs to reverse the sensitivity that the *pbs2Δ* mutant shows to tunicamycin. It was observed that the presence of the Hog1p and Ssk2/22p scaffold domains in Pbs2p are essential to allow growth in tunicamycin, while the Sho1p scaffold domain and the kinase domain were dispensable. We also found that a Hog1p mutant lacking its Pbs2p binding domain does not rescue the sensitivity shown by a *hog1Δ* strain. Furthermore, we found that in the presence of tunicamycin there is a strong interaction between Pbs2p and Hog1p, measured by reconstitution of the dihydrofolate reductase (DHFR) activity in a protein-fragment complementation assay (PCA). Finally, we were interested in determining the subcellular localization of Pbs2p under tunicamycin exposure. Using a GFP tagged version, we found that Pbs2p forms extranuclear aggregates that in some cases co-localize with an ER tracker. This project was supported by CONACYT project number 166734 and PAPIIT (DGAPA, UNAM) project number IN206513. MHE received a fellowship from CONACYT and a special support from PAEP-CEP, UNAM.

PS12-9: Arsenic directly binds and activates the yeast AP-1-like transcription factor Yap8

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The AP-1 like transcription factor Yap8 (also called as Acr1 and Arr1) is critical for arsenic tolerance in *Saccharomyces cerevisiae* [1]. Likewise, the Yap8 orthologue in *Kluyveromyces lactis* senses and responds to multiple stress signals including arsenic [2]. However, the mechanism by which Yap8 proteins sense the presence of arsenic and activate the transcription of detoxification genes is not yet known. Here, we demonstrate that Yap8 directly binds to trivalent arsenite *in vitro* and *in vivo*. Genetic and biochemical data pinpoint that critical cysteine residues in Yap8 form an essential binding site with arsenite. Arsenite binding by Yap8 does not require any additional yeast protein. Yap8 is neither regulated at the level of localization nor at the level of DNA

binding. Instead, our data is consistent with a model of Yap8 activation in which a DNA-bound version of the transcription factor acts directly as an arsenite sensor. Binding of arsenite to Yap8 triggers a conformational change that in turn brings about a transcriptional response. Thus, arsenite binding to Yap8 acts as a molecular switch that converts inactive Yap8 into an active transcriptional regulator. To our knowledge, this is the first report to demonstrate how a eukaryotic protein couples arsenic sensing to transcriptional activation.

[1] Wysocki, R. et al. (2004) *Mol. Biol. Cell* 15, 2049-2060; [2] Veide Vilg J., Kumar N.V., et al. (2014) *BBA Gene Reg. Mech.* 1839, 1295-1306.

PS12-10: New insights into regulation of Flo11p involved in biofilm formation

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Yeast biofilms are complex structures, in which cells are protected from hostile environments, including antifungals, host immune systems and other treatments. In *Saccharomyces cerevisiae*, Flo11p is a key protein of biofilm development. Many wild *S. cerevisiae* strains form structured (“fluffy”) colonies when they are able to produce Flo11p. In contrast, deletion of FLO11 gene results in smooth colony formation (*Fungal Genet Biol* 47: 1012-1022, 2010). Several pathways regulate Flo11p including the MAPK, TORC, Ras/cAMP/PKA, SNF1 and RIM101 pathways. After screening, we have focused on regulator ZLP2013, which positively regulates Flo11p. Deletion of the ZLP2013 gene blocks FLO11 gene expression and converts fluffy colonies to smooth ones. Simultaneously, it causes altered growth and cellular characteristics compared to wild type cells. Our results suggest that the control of FLO11 gene expression is highly complex and requires detailed investigation. The project is supported by GACR 15-08225S, SVV-2015-260209 and Biocev (CZ.1.05/1.1.00/02.0109).

PS12-11: Interaction between Ln3+ and *Saccharomyces cerevisiae* cells

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Trivalent lanthanide ions (Ln3+) have no intrinsic biologic role and they are not essential to life, but they have gained interest due to their potential utilization as optical probes and NMR contrast agents, especially in their complex coordinative forms. Apart from several therapeutic applications, Ln3+ are known as potent inhibitors of voltage and mechano-sensitive ion channels. Of all Ln3+, Gd3+ is widely used experimentally as an inhibitor of stretch-activated ion channels, but other Ln3+ have also been reported to have similar actions. The mechanisms of Ln3+ action are not entirely clear, but it is widely accepted that this inhibitory activity is the result of the similarity in Ln3+ cationic radii with that of Ca2+. Nevertheless, the specificity of the interaction between Ln3+ and the ion channels is questionable, as the Ln3+ bind with high affinity to phospholipids, thus affecting non-specifically the physical characteristics of the lipid bilayer and consequently altering the conformation of membrane-bound proteins. Although the biologic and medical importance of Ln3+ based on their similarity to Ca2+ is well recognized, systematic studies on Ln3+ accumulation or on how Ln3+ affect Ca2+ transport across the plasma membrane are currently lacking. In this study we make use of the eukaryotic model *Saccharomyces cerevisiae* to investigate the correlation between Ln3+ accumulation (whole series), their toxicity and their capacity to block the exogenous stress-induced Ca2+ influx into the cytosol. Using a haploinsufficiency assay, we found that Ln3+ toxicity can also be explained by Ln3+ binding to intracellular Ca2+ channels, such as Yvc1.

PS12-12: Different lifestyles are reflected in yeast colony differentiation

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Yeast colonies have become an excellent model for investigation of processes involved in cell differentiation and development of specific cell types, which acquire certain specific properties and functions according to their localization within the colony [1]. Two major types of colonies of yeast *Saccharomyces cerevisiae* could be classified according to their architecture: smooth colonies often formed by laboratory strains and structured biofilm colonies, often formed by wild strains. Yeast cells can switch between these two colony types

(representing two different yeast life-styles) according to growth conditions [2]. Smooth and structured colonies differ in many parameters including presence and localization of different types of differentiated cells that fulfill different functions. Examples are localization of cell subpopulations that actively divide or resting starving cells and presence of cells that produce extracellular matrix (which is completely absent in smooth colonies). On the other hand some other developmental characteristics are preserved in both colony types. Hence, both types of colonies pass through similar developmental phases characterized by changes of external pH from acidic to alkali and by production of ammonia during the alkali phase. Spatiotemporal analysis of presence of cells producing major selected marker proteins and regulators typical of specific cell types that have been identified in smooth colonies (U and L cells, [3]) allowed us to identify similar subpopulations in structured biofilm colonies. Differences in properties and in localization of these subpopulations as well as the first view in features that differ between both types of colonies and could represent differences connected with different life-style of wild and laboratory yeast strains will be presented. This work was supported by GACR 13-08605S and by BIOCEV (CZ.1.05/1.1.00/02.0109).

[1] Palkova Z, Wilkinson D, Vachova L (2014) FEMS Yeast Res 14, 96–108; [2] Stovicek et al (2010) Fungal Genet Biol 47, 1012-22; [3] Cap M, Stepanek L et al. (2012) Mol Cell 46, 436-48.

PS12-13: Transcription factors regulating ATO gene expression during development of yeast colonies

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Yeast colonies are complicated structures that develop both in time and in space. During the development colonies become partitioned into different sub-populations of cells having different physiological and morphological characteristics. In our laboratory, we have recently described two major sub-populations that are formed in the alkali period of colony development that is characterized by production of volatile ammonia: L-cells forming lower layers and U-cells localized to upper layers within the colonies (Mol Cell 46: 436–48, 2012). With the aim of identifying the regulatory network involved in colony differentiation and formation of specialized cell types, we focused on identification of regulators involved in expression of genes of the ATO family (coding for proteins Ato1p, Ato2p, Ato3p). Expression of all three ATO genes is strongly induced exclusively in U-cells. Using high-throughput screening of colonies formed by *Saccharomyces cerevisiae* strains carrying deletions of genes for selected transcription factors and fluorescently labeled Ato proteins (Ato-GFP), we identified several transcription factors which either activate or repress production of Ato proteins. Using spectroflurometry combined with *in vivo* visualization of production of specific Ato-GFP labeled proteins *in situ* within the colonies, we showed the specific effects of identified regulators in different cell subpopulations. The project is supported by GACR 13-08605S, SVV-2015-260209 and Biocev (CZ.1.05/1.1.00/02.0109).

PS12-14: Calcium signaling mediates the response to copper toxicity in *Saccharomyces cerevisiae* cells

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Essential heavy metals (Co, Cu, Fe, Mn, Ni, Zn) have been in the prime light of basic and applied research due to their dualistic action upon living organisms, being necessary in minute amounts for the normal metabolism but getting toxic when present in concentrations higher than the physiological levels. Other metals (e.g. Ag, As, Cd, Hg, Pb, Ln) do not have metabolic significance, but they can be highly toxic due to non-specific binding to cell components or by interfering with the normal metabolism of other metals. To respond to metal surpluses, cells have developed intricate ways of defense against the excessive metallic ions. To understand the ways in which cells sense the presence of toxic concentrations of metals, the involvement of Ca²⁺ in the response to high Mn²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, or Hg²⁺ was investigated in *Saccharomyces cerevisiae* cells. It was found by our group that the yeast cells responded through sharp increase in cytosolic Ca²⁺ when exposed to high Cd²⁺, and to a lesser extent to Cu²⁺, but not to Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, or Hg²⁺ [1]. In the present study we focused on investigating the role of Ca²⁺ in mediating the cell response to high concentrations of Cu²⁺. It was

found that the cell exposure to high Cu²⁺ determined broad and prolonged Ca²⁺ waves into the cytosol which showed a different pattern from the Ca²⁺ pulses induced by high Cd²⁺. The mechanisms of Ca²⁺-dependent response to surplus Cu²⁺ are discussed and the cell possibilities to discriminate between Ca²⁺-mediated adaptation to Cu¹⁺ or Cu²⁺ are presented.

[1] Ruta et al. (2014) *FEBS Lett.* 588, 3202-3212

PS12-15: Engineering yeast hexokinase 2 for improved tolerance toward xylose-induced inactivation

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Hexokinase 2 (Hxk2p) from *Saccharomyces cerevisiae* is a bifunctional enzyme with a catalytic function as well as an important regulatory function in the glucose repression signal. In the presence of xylose Hxk2p is irreversibly inactivated through an autophosphorylation mechanism, affecting all functions. Consequently, the ability to regulate expression of genes involved in sugar transport and fermentative metabolism is impaired. The aim of the study was to obtain new Hxk2p-variants, immune to the autophosphorylation, which potentially can restore the repressive capability closer to its nominal level. In this study we have constructed the first condensed, rationally designed combinatorial library targeting the active-site in Hxk2p. We combined protein engineering and genetic engineering for efficient screening and identified a variant with 64% higher catalytic activity in the presence of xylose. This variant is expected to be a key component for increasing the productivity of recombinant xylose-fermenting strains for bioethanol production from lignocellulosic feedstocks.

PS12-16: The rare glutamine tRNACUG is required for nitrogen catabolite repression-sensitive Gln3 localization

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A leucyl-tRNA synthetase pathway regulates TorC1 kinase activity and its downstream regulation of protein synthesis, a major consumer of nitrogenous precursors. TorC1 activity also regulates Gln3 and Gat1, the transcription activators of the catabolic genes whose products generate those precursors. Paradoxically, Gln3 isn't demonstrably regulated by the leucyl-tRNA synthetase pathway or Gtr-Ego-dependent TorC1 activation. A major component of Gln3 and Gat1 regulation is the control of their localization. In excess nitrogen Gln3 and Gat1 are sequestered in the cytoplasm in a Ure2-dependent manner. They become nuclear and activate transcription when preferred nitrogen source availability decreases or only poorly used nitrogen sources are available. Long-term nitrogen starvation and treating cells with the glutamine synthetase inhibitor methionine sulfoximine (Msx) also elicit nuclear Gln3 localization. The connection of Gln3 regulation and glutamine synthesis prompted us to investigate the effects of a glutamine tRNA mutation sup70-65 on Gln3/Gat1 localization. Nuclear Gln3 localization elicited by short- and long-term nitrogen starvation, growth in proline medium, Msx or rapamycin treatment or a ure2 deletion requires unaltered glutamine tRNACUG. Alteration of this rare tRNA, the sup70-65 mutation is epistatic to a ure2 deletion, suggesting tRNACUG may act downstream of Ure2. Nuclear Gat1 localization exhibits a tRNACUG requirement for its response to short-term nitrogen starvation, growth in proline medium or a ure2 deletion, but not for its response to rapamycin. These observations demonstrate the existence of a nitrogen-responsive, glutamine tRNA-dependent component participating in the control of Gln3 and Gat1 localization and their downstream regulation of nitrogenous precursor generation. Since Gln3 and Gat1 localization isn't demonstrably controlled by the leucyl-tRNA synthetase-TorC1 activation pathway, these data suggest that a second tRNACUG-dependent component is required likely downstream of Ure2 to achieve overall nitrogen-responsive regulation. We also demonstrate that Gat1 localization does not respond to long-term, Sit4-dependent nitrogen starvation. Supported by NIH grant GM-35642.

PS12-17: Gtr-Ego complex components participate in nuclear Gln3 localization, but not its cytoplasmic sequestration

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Vam6, Gtr1/2, and Ego1/3 are required for leucine-dependent TorC1 kinase activation which is central to nitrogen-responsive regulation. However, Gln3, a nitrogen-responsive transcription activator, does not respond to leucine-dependent TorC1 activation. In nitrogen excess, Gln3 is cytoplasmic and Gln3-mediated transcription minimal, whereas in nitrogen limitation, starvation, or following rapamycin treatment, Gln3 is nuclear and transcription greatly increased. Increasing evidence demonstrates nitrogen-responsive intracellular Gln3 localization is subject to multiple modes of regulation. To ascertain whether the Gtr-Ego complexes participate in the regulation of Gln3, we determined the requirements of Gtr1/2 and Ego1/3 proteins for nuclear localization and cytoplasmic sequestration of Gln3 in response to nitrogen excess, starvation or limitation. We show that Gln3 is sequestered in the cytoplasm of gtr1, gtr2, ego1 and ego3 deletions either long-term in logarithmically glutamine-grown cells or short-term after re-feeding glutamine to nitrogen-limited or -starved cells; GATA factor-dependent transcription was also minimal. However, in all of the deletion mutants except the gtr1 deletion, nuclear Gln3 localization elicited by nitrogen limitation or starvation is adversely affected. These data indicate that a Gtr-Ego-independent nitrogen-responsive mechanism exists to sequester Gln3 in the cytoplasm and suggests the above proteins likely possess additional functions beyond those associated with TorC1 activation. Support NIH GM-35642, COCOF and FRFC 2.4547.11.

PS12-18: Features and gene expression typical of structured yeast colonies

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In natural settings yeast *Saccharomyces cerevisiae* prefer to grow in the form of multicellular populations such as structured biofilm colonies. Existence within such structures gives the cell population better prospects to survive in hostile environment. For this the wild yeast strains evolved a number of protective strategies [1]. When meet nutrient surplus some cells switch off most of the protective mechanisms during the process called domestication [2] and start to form smooth colonies. The domestication can be reversed under adverse conditions when feral subclones start to appear that are able to form colonies with wild type-like morphology of biofilm colonies [3]. We compared physiology of wild, domesticated and feral strains by different approaches including transcriptomics. The feral strain forms colonies resembling biofilm colonies of wild strain in numerous aspects including restoration of the major features that are switched off during the domestication such as formation of extracellular matrix, production of Flo11p adhesin and ability to absorb high amount of water. Transcriptomic analysis identified the main functional groups of genes induced in colonies with “structured” morphotype, including genes linked to cell wall remodeling and plasma membrane characteristics as well as genes involved in signaling cascades. This work was supported by GACR 13-08605S and by BIOCEV (CZ.1.05/1.1.00/02.0109).

[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.

Poster Session 13: Yeast as a model in nutritional studies**PS13-1: A synthetic medium optimized for growth and a minimal medium for fermentation in yeasts**

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YPD medium and synthetic media containing Yeast Nitrogen Base (YNB), which was formulated in the early 1950's, are commonly used for yeast research and industrial protein production. It is known that the synthetic media do not show similar growth curve compared to the nutrient-rich YPD medium. For alcohol fermentation, complex media containing raw materials such as molasses and malts are used. If raw materials are used, necessary and sufficient nutrients cannot be controlled for efficient fermentation. The problem is that the traditional synthetic medium is not completely formulated for optimal growth and fermentation in yeasts. Some materials may be unnecessary or too much and others may be too low. Therefore, we have tried to identify necessary and sufficient nutrients in yeast growth. In the initial study of synthetic medium formulation, we searched nutrients that support growth of the yeast *Saccharomyces cerevisiae* prototrophic strain but failed. Then, we thought that amino acids might be important for growth. Therefore, every amino acid was mixed at various concentrations in various combinations and the growth of *S. cerevisiae* was monitored with shaking in Biorecorder, an automatic OD monitor. Finally, we obtained a formula for medium that showed similar growth to YPD, which we named AYD. The AYD contains all amino acids and some of the vitamins and minerals contained in YNB. AYD also showed similar growth to YPD in other yeast species. From this AYD medium, we tried to identify nutrients necessary for ethanol fermentation by using Fermograph, an automatic CO₂ monitor. For ethanol fermentation, only few vitamins and minerals are required, which we named ATD. Surprisingly, calcium ion was specifically required for high-temperature fermentation in the yeast *Kluyveromyces marxianus*.

PS13-2: Cadmium induces the activation of cell wall integrity pathway in budding yeast

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MAP kinases are important signaling molecules regulating cell survival, proliferation and differentiation, and can be activated by cadmium stress. In this study, we demonstrate that cadmium induces phosphorylation of the yeast cell wall integrity (CWI) pathway_MAP kinase Slt2, and this cadmium-induced CWI activation is mediated by the cell surface sensor Mid2 through the GEF Rom1, the central regulator Rho1 and Bck1. Nevertheless, cadmium stress does not affect the subcellular localization of Slt2 proteins. In addition, this cadmium-induced CWI activation is independent on the calcium/calcineurin signaling and the HOG signaling pathways in yeast cells.

PS13-3: Specific analogs uncouple transport, signaling, oligo-ubiquitination and endocytosis in an amino acid transceptor

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The yeast amino acid transceptor Gap1 functions as receptor for signaling to the PKA pathway and as nitrogen-limitation induced transporter, undergoing amino acid induced oligo-ubiquitination and endocytosis. We have identified specific amino acids and analogs, which uncouple signaling, transport, oligo-ubiquitination and endocytosis in nitrogen-starved cells. L-Lysine, L-histidine and L-tryptophan are transported like other amino acids but do not trigger signaling. Unlike L-histidine, L-lysine induces oligo-ubiquitination but almost not endocytosis. The non-transported signaling agonist, L-Leu-Gly, induces both oligo-ubiquitination and endocytosis. The non-transported, non-agonist for signaling, L-Asp- γ -L-Phe, which acts as competitive inhibitor of transport, induces oligo-ubiquitination but no discernible endocytosis. Transported, non-metabolizable signaling agonists, β -alanine and D-histidine, are strong and weak inducers of endocytosis, respectively, both causing Gap1 oligo-ubiquitination. These results show that a molecule can be transported by a transceptor without triggering signaling or substantial endocytosis, and that oligo-ubiquitination and endocytosis do not require signaling, nor transport or metabolism. Oligo-ubiquitination is required, but not sufficient for triggering endocytosis. We also demonstrate intracellular cross-induction of endocytosis of transport-defective Gap1 by ubiquitination- and endocytosis-deficient Gap1. Our results suggest that in transceptors different substrates provoke different conformational changes during transport and that signaling, oligo-ubiquitination and endocytosis each involve different conformational changes.

Poster Session 14: Systems biology of yeast**PS14-1: Pathway transplantation into yeast as a model for human disease****Neta Agmon, Jef D. Boeke***Institute for Systems Genetics, NYU Langone Medical Center, USA*

Decades of research have led to the development of a numerous 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 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. It is a highly conserved pathway from yeast to humans. In humans there are >20 disorders associated with the pathway that have a variety of symptoms. Importantly, most of these diseases lack any established treatment. We propose to use yeast for expressing human mutant alleles of human diseases in order to study their effect on the cell’s entire metabolic network 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 engineered a yeast strain “humanized” for the de novo adenine biosynthesis pathway. Deleted all of the yeast genes involved in the pathway and complemented them using a neochromosome expressing their human counterparts under the transcriptional control of their cognate yeast promoters and terminators. The “humanized” yeast strain shows growth in the absence of adenine in the medium, indicating complementation of the yeast pathway by the human one. We will thus ultimately attempt to swap all 26 genes in the entire network including the de novo guanine and salvage pathways, producing yeast with a completely “humanized” purine metabolic network. We are also introducing mutations from patients into the human genes to examine their effect on the “humanized” strain. These will than be used for analyzing their effect on the entire cell network in a variety of yeast based high-throughput methods as well as screening for new drugs tailored for the specific mutations examined. Surprising results suggest that certain missense mutations presumed to underlie human disease conditions have no impact on adenine biosynthesis in yeast. This could be explained by either a second function for the affected protein or an incorrect interpretation of a mutation. Finally, the Purine metabolic network can serve as a proof of principle for our ability to take human diseases and its associated pathway/gene network and establish a yeast model for the disease.

PS14-2: Increased mitochondrial metabolism in sake yeast**Gennaro Agrimi¹, Maria C. Mena¹, Kazuki Izumi², Isabella Pisano¹, Lucrezia Germinario¹, Hisashi Fukuzaki², Lars M. Blank³, Hiroshi Kitagaki^{2,4}, Luigi Palmieri¹**¹*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy;*²*Department of Environmental Sciences, Faculty of Agriculture, Saga University, Saga 840- 8502, Japan;*³*Institute of Applied Microbiology - iAMB, ABBt – Aachen Biology and Biotechnology Department, RWTH Aachen University, 52074 Aachen, Germany; ⁴Department of Biochemistry and Applied Biosciences, United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan*

The *S. cerevisiae* strain Kyokai no. 7 (K7) is one of the most extensively yeasts for the industrial sake production and has been employed as a model sake yeast in numerous genetic and biochemical studies. Our data show that K7 displays a high mitochondrial metabolism as compared to the reference laboratory strains CEN.PK 113-7D and BY4742. In the experimental conditions used in our work (synthetic medium supplemented with 0.5% glucose; early exponential phase), K7 shows a two fold higher pyruvate flux towards mitochondria and an almost four fold increase of the TCA cycle activity compared to CEN.PK 113.7D as determined by ¹³C-Metabolic Flux Analysis. A previously developed pyruvate undersecreting sake yeast obtained by isolating a strain (TCR7) tolerant to ethyl α-transcyanocinnamate, an inhibitor of pyruvate transport into mitochondria, displays an even higher pyruvate flux into mitochondria even though a Real-Time PCR analysis of the expression level of the 3 Mitochondrial Pyruvate Carrier (MPC) subunits, did not reveal any significant differences between K7 and TCR7. When shifted from aerobic to anaerobic conditions, sake yeast retained a branched mitochondrial structure for a longer time than the laboratory strains. Although mitochondrial metabolism decreases upon transfer to mostly anaerobic conditions similar to those found in industrial fermentations, residual mitochondrial activity and its decrease in the course of the brewing process can be a key factor in determining the organic acid profile during fermentation.

PS14-3: Systematic identification and correction of annotation errors in the genetic interaction map of *Saccharomyces cerevisiae*

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One of the main goals of systems biology is to achieve all-encompassing knowledge about an organism's genetic components and their interactions. The yeast mutant collections, composed of yeast strains with a single, defined mutation, allow the systematic exploration of genomic organization and function. In a decade-long effort, using sophisticated genetic manipulations, a large database describing all possible genetic interactions in yeast is being created. These data constitute an important resource used by many researchers and serves to map genetic pathways and to assign function to unknown genes. We have previously described the neighboring gene effect (NGE), a phenomenon by which the deletion of one gene affects the expression of an adjacent gene along the genome. Here we analyzed a dataset of ~6,000,000 double gene knockout measurements. Focusing on an observed set of ~90,000 negative genetic interactions, we found that more than 10% are incorrectly annotated due to NGE. We developed a novel algorithm, Genetic Interaction Neighboring Gene Effect Recovery (GINGER), to identify and correct erroneous interaction annotations. We validated the algorithm using a comparative analysis of genetic interactions from *S. pombe*. We further showed that our predictions are significantly more concordant with diverse biological data compared to their mis-annotated counterparts. The GINGER algorithm successfully corrects interactions that are mis-annotated due to NGE, uncovering ~9,500 new genetic interactions.

PS14-4: Deciphering the molecular mechanisms underlying robustness to protein overproduction

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Why are proteins harmful when expressed in excess? Here, we systematically investigate the impact of genetic variation and the environment on protein overproduction costs in *Saccharomyces cerevisiae*. By integrating genome-wide genetic interaction and environmental stress screens, we identified three main mechanisms buffering the fitness costs of protein burden. Overproduction of unneeded proteins occupies ribosomes which could better be used for the translation of native proteins (ribosome occupancy) and wastes cellular resources (energy conservation). In addition, according to what we term the chaperone overload hypothesis, the cost of unneeded protein production is also strongly determined by the translation-associated protein folding machinery. When protein folding capacity is compromised, protein overproduction disrupts the global balance of proteostasis and leads to the accumulation of aggregated proteins. Our work demonstrates the existence of multiple key genes that buffer protein overload and thereby may facilitate major changes in genomic expression during evolution.

PS14-5: Systems biology research infrastructure and the yeast research community

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A deeper understanding of complex biological functions requires an iterative process of integration and structuring of hypothesis-driven approaches and omics data based on the use of computational models, to allow researchers to simulate, analyze and predict the behavior of the system model investigation. This systems biology approach is strongly multidisciplinary and it is going to be supported in Europe by an ESFRI Research Infrastructure, ISBE, presently under construction. ISBE will provide an open-access to state-of-the-art facilities, data, models, tools and training, as well as fostering project collaborations with experimental and computational teams. ISBE will also make possible to integrate both reductionist and systems biology approaches in many projects, improving the attractiveness of the results (i.e. papers, patents) for journals, funders, industries.

Budding yeast is an excellent model organism for systems biology studies: the construction of a Yeast Whole-Cell Model will be able to promote a better understanding of basic cellular functions, given the environmental conditions, and also in mammalian cells. SYSBIO - Centre of Systems Biology (www.sysbio.it) is the Italian Institute for Systems Biology and ISBE Associate partner. SYSBIO is deeply engaged with Yeast Systems Biology, working on cell cycle, signaling, metabolism, cell death, aging, synthetic biology and bioprocesses, using yeast as both major player and model system. SYSBIO is ready to develop collaborative projects of Systems biology on yeast and to offer open access to both our modeling skills and our state-of-the-art facilities, like the new metabolomics lab.

PS14-6: Characterization of a yeast strain able to utilize glutamate as sole carbon and nitrogen source

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Budding yeast *Saccharomyces cerevisiae* is classified as “Krebs negative”, since it is unable to growth on media containing as carbon sources the di- or tricarboxylic acids of TCA cycle like 2-oxoglutarate or citrate. Glutamate constitutes a good nitrogen source for *S. cerevisiae*, as it can sustain high growth rate when added to the medium. Glutamate is imported by the yeast, and subsequently deaminated to 2-oxoglutarate with the formation of NADH by the action of the cytosolic enzyme glutamate dehydrogenase (Gdh2). Given the possibility for 2-oxoglutarate to enter TCA cycle and the formation of reducing equivalents, glutamate could in principle constitute a unique carbon and nitrogen source capable to sustain growth. As a matter of fact, *S. cerevisiae* results unable to growth in media based on glutamate alone, confirming that Krebs negative phenotype was not related to transport problems. Since examples are known of Krebs positive yeast, we decided to study the phenomenon with an holistic approach, trying to identify the factors that control the growth on such substrates. With the application of an evolutionary strategy we could isolate some clones, coming from three independent serial transfers, able to form colony on glutamate plates. Four clones have been characterized in shake flasks, revealing differences in their growth rate and in the final biomass reached. In particular, all the clones growth with very high duplication times, ranging from 29 to 47 hours, while growth on glucose resulted similar to the wild type. As regard to the final biomass, we could observe a complete glutamate depletion only in bioreactor, where pH control and oxygenation were optimal but those parameters didn't affects the growth rate. In order to unravel the changes in metabolism that confer to the mutants the ability to growth on glutamate we have currently underway sequence, transcriptomic and metabolomic analysis of our clones. We believe that studies on mutant's metabolism will be a useful complement to current profiling and modelling in the wild type, aimed to the elucidation at system cell level of glutamate metabolism in yeast.

PS14-7: An experimental design approach for engineering carbon metabolism in the yeast *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is an attractive host for industrial production of biofuels and platform chemicals. It is generally regarded as safe, is scalable and is used within current industrial infrastructure. Its predominant carbon flux to ethanol is however a significant metabolic engineering challenge for the biosynthesis of alternative products; rather it is desirable to divert this flux towards target compounds. A workflow has been designed for generation of a multiple alcohol dehydrogenase (ADH) gene knockout library using the dominant, counter-selectable amdSYM deletion cassette. Importantly, this is applicable to industrial strains of *S. cerevisiae* and does not leave short repetitive elements in the genome which generate genetic instability in this species. Moreover, this library allows assessment of the Design of Experiments (DoE) methodology for metabolic engineering of *S. cerevisiae*. To this end, an industrially relevant *S. cerevisiae* ADH isozyme perturbation toolbox has been constructed. A high-throughput mini-stat system has been developed in order to comparatively evaluate the performance of the toolbox. A customised expression vector has been designed to express heterologous pathways for production of target compounds in the ADH knockout toolbox. Production of target compounds is being evaluated under various environmental conditions using a DoE methodology, providing a structured and

detailed assessment of the strains' fermentation performance, trade off analysis and industrial applicability.

PS14-8: Intrinsic biocontainment: Multiplex genome safeguards combine transcriptional and recombinational control of essential *Saccharomyces cerevisiae* genes

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Biocontainment may be required in a wide variety of situations such as work with pathogens, field release applications of engineered organisms, and protection of intellectual properties. Here, we describe the control of growth of the brewer's yeast, *Saccharomyces cerevisiae*, using both transcriptional and recombinational "safeguard" control of essential gene function. Practical biocontainment strategies dependent on the presence of small molecules require them to be active at very low concentrations, rendering them inexpensive and difficult to detect. Histone genes were controlled by an inducible promoter and controlled by 30 nM estradiol. The stability of the engineered genes was separately regulated by the expression of a site-specific recombinase. The combined frequency of generating viable derivatives when both systems were active was below detection ($<10^{-10}$), consistent with their orthogonal nature and the individual escape frequencies of $<10^{-6}$. Evaluation of escaper mutants suggests strategies for reducing their emergence. Transcript profiling and growth test suggest high fitness of safeguarded strains, an important characteristic for wide acceptance.

PS14-9: A stochastic hybrid model of the G₁/S transition *Saccharomyces cerevisiae*

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A stochastic hybrid model of the molecular network controlling the G₁/S transition for the budding yeast *Saccharomyces cerevisiae* is here presented. The network encompasses major molecular players, dealing with a chain of events leading to expression of Cyclins *Cln1*, *Cln2* and activation of Cyclin Dependent Kinases *Clb5,6* and *Cln1,2*, responsible for the onset of the S-phase and for the emergence of the bud, respectively. A pivotal role is played by *Whi5*, inhibitor of *SBF* transcription factor, whose multisite phosphorylation is proven to be responsible of the synchronization of the many (above 200) G₁/S genes controlled by *SBF*. Differently from other molecular dynamics (basically ruled by mass-action law) the *SBF/Whi5* interplay is modeled by means of a discrete event stochastic model. It is shown that such a molecular model is able to account for the many experiments available in the literature: it allows the setting of the critical size without assuming a linear growth rate [1]; it explains cell size variability as a function of G₁ phase duration [2]; it explains mutant framework such as SBF overexpression [3]; it can also be extended to account for alpha-factor administration [4].

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PS14-10: Enzymes with multiple roles: exploring the molecular basis of pleiotropy in yeast

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Enzyme malfunction can be linked to different, sometimes unexpected, phenotypes. Whether this reflects simply a perturbed catalytic activity or additional molecular functions of the polypeptide has not been systematically addressed. Here, we ask to what extent enzyme phenotypes depend on their associated catalytic activities. We introduce a systematic strategy based on the comparison of high-resolution phenotypic profiles of gene-knockout and catalytic-site mutant strains grown under different environmental conditions. This experimental strategy

allowed us to explore the catalytic-independent phenotypes of enzymes and the identification of additional, previously unknown, molecular functions of proteins. We focused on a set of well-characterized yeast enzymes of amino acid biosynthetic metabolism and identified new cases of enzymes with more than one molecular function. We will present specific examples which suggest that yeast enzymes mediate cellular crosstalks of metabolism and general stress control by means of such "moonlighting" activities. Further exploration of catalytic-independent phenotypes and protein multifunctionality will contribute to a better understanding of cellular systems, from cell metabolism and gene-function annotation, to single-gene disorders, pleiotropy, and complex disease.

PS14-11: Understanding low pH tolerance in *S. cerevisiae* using adaptive laboratory evolution

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Tolerance of microbial hosts to low pH is a major consideration in the industrial production of lactic acid, a platform chemical used for the synthesis of polylactic acid (a bioplastic). Lactic acid fermentation at a pH lower than its pKa (3.86) is preferred in order to obtain high amounts of the undissociated form of the acid for polymerisation into polylactic acid. However, the growth of yeast (a more preferred microorganism for this process) is severely inhibited at this low pH necessitating the need to neutralise the growth media leading to high recovery costs. Therefore, obtaining low pH resistant yeast strains will be crucial in making this process cost-effective. To this end, we have used an adaptive laboratory evolution approach to select strains of *Saccharomyces cerevisiae* with improved growth at pH 2.8. By re-sequencing the whole genome and total RNA of evolved mutants followed by using several omics tools, important mutations and biological processes that selectively increase resistance to low pH in these mutant strains have been identified. Ultimately, we hope to use this information to generate more robust lactic acid producing yeast strains which can yield higher product concentrations and greatly improve the process economics.

PS14-12: Redox and energetic trends during exponential growth and diauxic shift in *saccharomyces cerevisiae*

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In unicellular microorganisms like *Saccharomyces cerevisiae* there is a strict relation among cellular metabolism, growth energy and redox balance. In this organism, due to the lack of a transhydrogenase activity, the surplus of NADH generated by catabolic and anabolic reactions has to be recycled by the respiratory chain or with the formation of glycerol and ethanol. Moreover the balance of energetic cofactors is quite complex. In fact, ATP and ADP concentration depends on the relative rate of catabolic fluxes and on the efficiency and rate of anabolic reactions. In turn, variations in ATP and ADP concentrations can deeply impact on the metabolic pathways, and in many case the cell prefers the disposal of part of the energy in futile cycles to the risk of an irreversible block of its metabolism. AMP concentration also plays a role as an indicator of the cell energy state, through the action of Snf1, a central member of the highly conserved AMP-activated protein kinase cascade, activated by increases in the AMP:ATP ratio. Once activated the enzyme switches off ATP-consuming anabolic pathways and switches on ATP-producing catabolic pathways. The Snf1 complex in yeast is activated in response to the stress of glucose deprivation, as in the case of standard batch growth on glucose, where cells undergo substantial physiological and metabolic changes. During the high growth rate phase, metabolism is mainly glycolytic with ethanol accumulation. Once glucose is depleted, the cells slow down, increase their respiration and activate gluconeogenesis and glyoxylate cycle, in order to consume the previously produced ethanol. Despite the widespread relevance of the switch from glycolysis to gluconeogenesis, we still lack a system-level understanding about the regulatory mechanisms that govern this transition. The dynamics of redox cofactors (NAD(H) and NADP(H)) and energetic molecules (ATP, ADP, AMP) along the transitions of a standard diauxic growth, have not yet been systematically studied. Most of the cofactors are, in fact, present at very low levels in the cell and some of them show a low stability. Aim of this work is to collect these missing data about the redox and energetic balance, representing relevant constraints to be imposed to a model of yeast growth and metabolism.

This information will allow to make better prediction of metabolic yield and flux distributions, under different cultural conditions.

PS14-13: Poligenic analysis of low temperature adaptation in wine yeast

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Many factors such as must composition, juice clarification, the temperature of fermentation or the yeast strain inoculated strongly affect alcoholic fermentation and aromatic profile of wine. With the effective control of fermentation temperature by the wine industry, low temperature fermentation (10 – 15 °C) are becoming more frequent due to the aim of producing white and “rosé” wines with more pronounce aromatic profile. Low temperatures increase not only the retention but also the production of some volatiles compounds. In these conditions greater concentration of aroma compounds are produced, such as esters that impart sweet and fruity aromas and lesser amounts of unpleasant compounds are produced, such as certain higher alcohols and acetic acid. Another interesting aspect is that low temperatures notably reduce the growth of acetic and lactic acid bacteria, facilitating the control of alcoholic fermentation. However fermentation at low temperature presents some disadvantages: reduced growth rate, long lag phase, sluggish or stuck fermentations. These problems can be avoided by selecting better-adapted yeasts to ferment at low temperature. Low temperature adaptation, as most enological traits of industrial importance in yeast, is a polygenic trait, regulated by many interacting loci. In order to address the genetic determinants of low temperature fermentation, we implemented a QTL mapping in the F12 offspring of two *Saccharomyces cerevisiae* industrial strains with a divergent phenotype in their performance at low temperature. We identified four genomic regions implicated in the fermentation process at low temperature in wine yeast. We found that subtelomeric regions play a key role in defining individual quantitative variation, emphasizing the importance of the adaptive nature of these regions in natural populations. Reciprocal hemizygosity analysis and deletion of the complete subtelomeric regions is underway to confirm the causative genes in the QTLs and understanding the underlying mechanism.

PS14-14: Evolution of robustness to protein mistranslation by accelerated protein turnover

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Translational errors occur at high rates, influence organism viability and the onset of genetic diseases. To investigate how organisms mitigate the deleterious effects of protein synthesis errors during evolution, a mutant yeast strain was engineered to translate a codon ambiguously (mistranslation). It thereby overloads the protein quality control pathways and disrupts cellular protein homeostasis. This strain was used to study the capacity of the yeast genome to compensate the deleterious effects of protein mistranslation. Laboratory evolutionary experiments revealed that fitness loss due to mistranslation can rapidly be mitigated. Genomic analysis demonstrated that adaptation was primarily mediated by large-scale chromosomal duplication and deletion events, suggesting that errors during protein synthesis promote the evolution of genome architecture. By altering the dosages of numerous, functionally related proteins simultaneously, these genetic changes introduced large phenotypic leaps that enabled rapid adaptation to mistranslation. Evolution increased the level of tolerance to mistranslation through acceleration of ubiquitin-proteasome mediated protein degradation and protein synthesis. As a consequence of rapid elimination of erroneous protein products, evolution reduced the extent of toxic protein aggregation in mistranslating cells. However, there was a strong evolutionary trade-off between adaptation to mistranslation and survival upon starvation: the evolved lines showed fitness defects and impaired capacity to degrade mature ribosomes upon nutrient limitation. Moreover, as a response to an enhanced energy demand of accelerated protein turnover, the evolved lines exhibited increased glucose uptake by selective duplication of hexose transporter genes. We conclude that adjustment of proteome homeostasis to mistranslation evolves rapidly, but this adaptation has several side-effects on cellular physiology. Our work also indicates that translational fidelity and the ubiquitin-proteasome system are functionally linked to each other and may, therefore, co-evolve in nature.

PS14-15: Genome-scale modeling helps to increase lipid accumulation in the oleaginous yeast *Yarrowia lipolytica***Martin Kavšek**, Govindprasad Bhutada, Sepp D. Kohlwein, Klaus Natter*Institute of Molecular Biosciences, University of Graz, Austria*

Yarrowia lipolytica is an oleaginous yeast that is extensively investigated for several biotechnological applications including citrate and single-cell oil production. If a highly accurate genome-scale reconstruction is available, genetic engineering of such metabolic pathways can be guided by computational modeling to speed up hypothesis-driven optimization towards higher productivities and yields. Based on a scaffold model of *S. cerevisiae* we reconstructed a new genome-scale model of *Y. lipolytica* and optimized it for use in flux balance analysis. The model, iMK735, shows an excellent agreement with experimental values for growth on different carbon sources and in lipid production phases. High rates of lipid synthesis depend on the continuous supply of high amounts of NADPH and acetyl-CoA. *In silico* expression of heterologous enzymes revealed promising alternative sources for these two compounds, which increased the maximum theoretical yields for triacylglycerol production. Based on these computational approaches, experimental strategies to genetically engineer *Y. lipolytica* for improved lipid accumulation are presented. Furthermore, we simulated different cultivation techniques to reduce citrate excretion and increase the lipid yield. We obtained an 80% increase in lipid content and a four-fold improvement of the lipid yield, as compared to standard conditions. Hence, iMK735 is a functional and accurate genome-scale model of *Y. lipolytica* that can be used for the analysis of metabolism and the effects of genetic interventions of this species. In this regard, iMK735 will be a helpful tool to gain additional insights into lipid production and storage of this alternative yeast.

PS14-16: Surveying triple mutant genetic interaction space in yeast**Elena Kuzmin**^{1,2}, Benjamin VanderSluis^{3,4}, Yiqun Chen², Matej Ušaj², Michael Costanzo², Chad L. Myers³, Brenda Andrews^{1,2}, Charles Boone^{1,2}¹*Molecular Genetics, University of Toronto, Canada;* ²*Donnelly Centre for Cellular & Biomolecular Research, Toronto, Canada;* ³*Computer Science & Engineering, University of Minnesota-Twin Cities, Minneapolis, USA;*⁴*Current address: Simons Center for Data Analysis, Simons Foundation, New York City, USA*

The global double mutant genetic interaction network is a valuable resource for understanding gene function and revealing key features of the global genetic interaction network. However, many phenotypes in simple eukaryotes as well as metazoans are likely influenced by more complex genetic interactions involving larger sets of genes and genetic variants. Thus, deciphering the genotype-phenotype relationship for an individual requires that we expand our focus beyond pair wise genetic interactions (digenic interactions) to include complex, higher-order genetic interactions involving more than two genes. In this study we used automated form of yeast genetics, called Synthetic Genetic Array (SGA) analysis, to construct double and triple mutants, and to measure genetic interactions by using colony size as a proxy for cell fitness. In total, we generated double and triple mutant genetic interaction profiles for over 150 singleton gene pairs comprising of essential and non-essential genes as well as pairs with mixed essentiality spanning all major biological processes in the cell. The resulting trigenic interaction network enabled us to develop a model that can predict trigenic interactions based on features characteristic of a digenic interaction network and estimate the extent of triple mutant interaction space in a eukaryotic cell. Furthermore, trigenic interactions involving *S. cerevisiae* homologs of *S. pombe* synthetic lethal pairs allowed us to probe the level of genetic interaction network conservation between distantly related species as evolutionary far apart from each other as worm and human. Thus, the general principles that emerge from mapping more complex genetic interaction networks should shed further light on how genes buffer one another as well as on the genetic mechanisms that underlie heritable diseases.

PS14-17: Quantitative multi-layer stress regulation analysis in yeast**Petri-Jaan Lahtvee**, Jens Nielsen*Chalmers University of Technology, Sweden*

Yeast is extensively used in various biotechnological processes with the global market reaching yearly into billions of dollars. However, to make bio-processes more cost efficient and more generally applied, well performing cell factories with increased production of precursor molecules and robustness towards stress factors

must be developed. In the current study, gradual increase of three different stress conditions (ethanol, osmolarity and temperature) were studied in the yeast *Saccharomyces cerevisiae* at constant dilution rate to understand the regulation of induced stress conditions without effects caused by decreasing specific growth rate. More than thirty chemostats were performed at constant dilution rate but due to varying stress conditions and levels, significant variations in specific glucose uptake rate were detected. The major common specific glucose uptake rate dependent change took place in mitochondria where mainly oxidative phosphorylation related genes and proteins showed strong specific glucose consumption rate-dependence. Latter was also the biggest group of genes that showed significant transcriptional regulation. Rather surprisingly, only relatively small overlap was determined at mRNA and protein levels between different stress conditions. As a common effect, elevated stress caused a decrease in biomass yield which was directly related to increased maintenance energy demand of the cells, however, effects for increased maintenance seemed to vary from one stress condition to another. General underlying energy dependent curve was discovered which also allows one to predict the on-set of overflow metabolism. Various integrative data analysis, including transcription factor analysis, indicated more similar response towards temperature and ethanol stress conditions, where protein turnover and membrane fluidity were mainly influenced. Osmotic stress showed more distinct patterns as oxidative stress pathways were activated. Gathered quantitative multi-layer data-sets together with integrated data analysis sheds light on the regulatory patterns and energy metabolism for each stress conditions separately and defines a general stress response in yeast. Additionally, it gives suggestions for metabolic engineering purposes to produce more robust cell factories.

PS14-18: RiboWaves: Simulating ribosome profiles in *Saccharomyces cerevisiae* transcriptomes

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Protein synthesis is a highly regulated process, whose controls affect the movement and the organization of the many ribosomes bound to the same mRNA (polyribosome), ultimately reshaping proteomes and phenotypes separately from mRNA levels. Yet, the contribution of regulation layers of translation and the possible role of ribosomes organization along the transcript are still largely unknown. Ribosome profiling, based on deep sequencing of ribosome protected mRNA fragments, has been extensively used to measure ribosome density along all mRNAs in the cell [1,2], providing unprecedented opportunities for the analysis of mRNA translation. Nevertheless, many issues arise both from the experimental [3] and analysis [4] pipelines used to extract the final data. The procedure is also expensive and time-consuming. We propose RiboWaves, a stochastic model that simulates translation of single ribosomes with codon resolution, taking as input mRNA sequences. RiboWaves is able to return ribosome positional densities and transcript-specific translation efficiencies, which can be directly compared with ribosome profiling outcomes. We applied our approach to *Saccharomyces cerevisiae* and human transcriptomes, considering organism-specific features such as codon usage bias and transcript-specific initiation rates. Comparing our simulations with a ribosome profiling dataset of exponentially growing *S. cerevisiae* [5], we isolated a population of transcripts with good agreement between predicted and experimental profiles, and a population with no correlation at all, pointing to translational controls not yet included in the model or to ribosome profiling biases. Overall, we found a statistically higher correlation in *S. cerevisiae* than in human datasets. We also compared predicted and experimental ribosome pause sites, corresponding to locations characterized by ribosome accumulation. Finally, we tried to include RNA binding proteins interactions in the model to understand how much their involvement improves simulation predictions. The model can be further applied to compare translation changes in different conditions and/or after specific cell treatments. Our results highlight the ultimate goal of RiboWaves, i.e. to better understand the role and the contribution of different regulation layers in shaping the complex process of translation.

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PS14-19: Network reconstruction and validation of the Snf1/AMPK pathway in baker's yeast based on a comprehensive literature review

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The SNF1/AMPK protein kinase plays a central role in energy homeostasis in eukaryotic cells. It is activated by energy depletion and stimulates processes leading to the production of ATP while it down-regulates ATP-consuming processes. The yeast SNF1 complex is best known for its role in glucose derepression, where it orchestrates the adaptation of metabolism to nutrient availability. Here, we report on a network reconstruction of the Snf1 pathway based on a comprehensive literature review. We reviewed the entire Snf1 literature and extracted mechanistic information about the pathway, which we formalised in the rxncon language. This machine readable network definition summarises the mechanistic knowledge of the Snf1 pathway, and was used to generate a bipartite Boolean model via the rxncon toolbox. We used this model and the known input/output relationships in the network to identify and fill gaps in the information transfer through the pathway, to produce a functional network model. The workflow presented here enables large scale reconstruction, validation and gap filling of signal transduction networks. It is analogous to but distinct from that established for metabolic networks. We demonstrate the workflow capabilities, and the direct link between the reconstruction and dynamic modelling. The Snf1 network is a knowledge resource for modellers and experimentalists alike, and a template for similar efforts in higher eukaryotes. Finally, we envisage the workflow as an instrumental tool for reconstruction of large signalling networks across Eukaryota.

PS14-20: Investigation of K2 toxin binding to yeast cell wall components

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Certain *Saccharomyces cerevisiae* strains contain different double-stranded RNA, encapsulated in virus-like particles, capable to secrete one of four types of killer toxins (K1, K2, K28, and Klus). These proteins are able to kill the nonkiller yeast, as well as yeast of other killer type, while the toxin-producing cells remain immune to their own or to the same type of toxin. The presumed mode of killer action involves two steps. The first step requires binding of the toxin to cell wall components, whereas the second step leads to the translocation and insertion of the toxin into the plasma membrane (for K1 and K2 toxin) or initiation of toxin endocytosis and subsequent DNA replication blockage (for K28). The mode of action of the Klus toxin is unknown. In this study, we have determined that K2 toxin saturates the yeast cell surface receptors in 10 minutes. The apparent amount of K2 toxin, bound to a single cell of wild type yeast under saturating conditions, was estimated to be 435–460 molecules. It was found that increased level of β-1,6-glucan directly correlates with the number of toxin molecules bound, thereby impacting the morphology and determining the fate of the yeast cell. We observed that the binding of K2 toxin to the yeast surface receptors proceeds in a similar manner as in case of the related K1 killer protein. It was demonstrated that the externally supplied pustulan, a poly-β-1,6-glucan, but not the glucans bearing other linkage types (such as laminarin, chitin, and pullulan) efficiently inhibits the K2 toxin killing activity. In addition, the analysis of toxin binding to the intact cells and spheroplasts confirmed that majority of K2 protein molecules attach to the β-1,6-glucan, when compared to the plasma membrane-localized receptors.

PS14-21: Exploration of conditional genetic interactions via barcode sequencing

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Our group has developed and applied methods for automated yeast genetics to produce large-scale networks of genetic interactions (GIs) under standard growth conditions [1]. The Synthetic Genetic Array (SGA)

methodology has also been used to explore how genetic interactions change in response to DNA damage and other stress conditions (e.g. [2,3]), but a systematic exploration of the condition-sensitivity of genetic interactions has not been undertaken. We are developing modifications of the SGA method to explore condition-specific changes in GI network structure and topology. A typical SGA analysis quantifies GIs using colony size measurements derived from high density single and double mutant arrays grown on agar plates. Using BarSeq [4,5], we performed pooled SGA analysis of a control gene and a test gene, *ura3Δ* and *bni1Δ* respectively for the entire yeast nonessential gene deletion collection grown in media containing methylmethane sulfonate (MMS), a DNA alkylating agent. The barcode frequency obtained in the Barseq SGA analysis correlate to result of a *bni1Δ* SGA analysis perform on agar plate under MMS, reinforcing that both methodology can be used to detect Gis. The GIs detected were enrich for gene ontology (GO) such as cell polarity ($p<0.001$), cell cycle progression ($p<0.003$) and chromatin remodelling ($p<0.01$) upon MMS treatment. We found that *bni1Δdot1Δ* strain reveals a conditional GI on *MMS*. *DOT1* is responsible for silencing chromatin and is involve in DNA repair. We assembled evidences that *BNI1* through chromatin remodelling have an impact on progression through *MAD2*-dependant checkpoint.

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PS14-22: Snf1/AMPK negatively regulates cAMP content and protein kinase A-dependent transcription in *Saccharomyces cerevisiae*

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Snf1 is a serine/threonine kinase required by the yeast *S. cerevisiae* to grow in nutrient-limited conditions and to utilize carbon sources alternative to glucose, as sucrose and ethanol [1]. Following nutrient deprivation Snf1 is activated and translocated to the nucleus where it can phosphorylate and inactivate the transcriptional repressor Mig1, causing the expression of over 400 genes [2]. Studying the involvement of Snf1 in the regulation of other signaling pathways we identified, through CoIP/MS experiments, the interaction between Snf1 and adenylate cyclase (Cyr1), the enzyme responsible for the synthesis of cyclic AMP (cAMP), activator of PKA. Furthermore, we demonstrated that in a Snf1-G53R strain, in which the kinase is constitutively activated, the expression of PKA-dependent genes is deregulated. We therefore hypothesized the existence of a new crosstalk mechanism between the Snf1 and PKA pathways, regarding which only the PKA regulation on the Snf1 pathway and the synergistic action of both kinases on common targets were known [3-8]. The sequence of Cyr1 presents five possible phosphorylation consensus sites, of which two are phosphoserines in vivo. The RAS Associating Domain of Cyr1, containing 2 putative Snf1 phosphorylation sites, was purified in *E. coli* and its in vitro phosphorylation by Snf1 was demonstrated. Moreover, in a Snf1-G53R strain we found a reduction of 50% of intracellular cAMP, compared to the wt. We therefore hypothesized that this reduction could be responsible for a reduced activation of PKA and, as a consequence, for the deregulation of the expression of PKA-dependent genes. Moreover, we performed in vivo mapping of phosphorylated sites of adenylate cyclase by mass spectrometry, identifying several sites, mainly in the regulatory domain of the protein, for which the phosphorylation is Snf1-dependent. In summary, our data suggest a new crosstalk mechanism, with a Snf1-dependent regulation of the PKA pathway. The Ras/cAMP/PKA pathway in budding yeast has been already modelled [9], and our work will serve to extend the model, integrating the action of the Snf1/AMPK pathway as a modulator.

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PS14-23: A Growth and cycle model for *Saccharomyces cerevisiae*Pasquale Palumbo^{1,2}, Marco Vanoni^{1,3}, Federico Papa^{1,2}, Lilia Alberghina^{1,3}¹SYSBIO, Centre of Systems Biology, Milan, Italy; ²IASI "A. Ruberti", National Research Council (CNR), Rome, Italy; ³Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

A low granularity model is proposed, aiming to describe the major features of cell growth and cell cycle in budding yeast *Saccharomyces cerevisiae*. Central for the construction of the Growth and Cycle Model (GCM) has been the presence of a large literature covering the description of cellular growth in steady-state and perturbed growth conditions in terms of ribosome and protein contents. As far as the cycle model, it complies the *Sizer* mechanism, a molecular machinery allowing a cell to “weigh” its mass before committing to a specific event, such as the G₁/S transition. The GCM is substantially composed of three modules: i) a dynamical cell growth module described by a set of ODEs, which represents the dynamics of synthesis and degradation of ribosomes and proteins; ii) a molecular triggering mechanism, linking cell growth to cell cycle, described by a set of ODEs detailing the dynamics of the growth-controlled activator *Cln3* and of its cognate inhibitor *Far1*; iii) a set of three consecutive timers (*T_{lb}*, *T₂* and *T_B*) that temporally account for the yeast cell cycle after the *Cln3/Far1* molecular machinery is triggered, underlying the length of the G₁ phase (including *T_{lb}* and *T₂*) and of the budded phase (timer *T_B*).

PS14-24: Multisite Whi5 phosphorylation synchronizes the activation of the G1/S regulon genesPasquale Palumbo^{1,2}, Marco Vanoni^{1,3}, Valerio Cusimano^{1,2}, Stefano Busti^{1,3}, Costanzo Manes^{2,4}, Lilia Alberghina^{1,3}¹SYSBIO, Centre of Systems Biology, Milan, Italy; ²IASI "A. Ruberti", National Research Council (CNR), Rome, Italy; ³Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy;⁴Department of Information Engineering, Computer Science and Mathematics, University of L'Aquila, L'Aquila, Italy

Whi5 inhibits transcription factor SBF and regulates the activation of the G₁/S regulon, leading to the onset of the budded phase in the yeast *Saccharomyces cerevisiae*. Previous work from our laboratory has shown that multisite phosphorylation of a regulatory protein is an effective molecular device able to produce a coherent response in the regulated function, when it involves hundreds of identical molecular players [1]. So we reasoned that the multisite phosphorylation of *Whi5* may constitute a relevant player of the regulatory network controlling the G₁/S transition. A stochastic hybrid mathematical model was constructed and its computational analysis clearly shows that the multi site phosphorylation of *Whi5* is indeed the core mechanism providing the synchronization in the activation of the many (above 200) genes controlled by *Whi5*. To this end, a crucial role is played by the proper partition among “functional” and “decoy” phosphorylation sites available on the inhibitor structure. Main results have been supported by laboratory experiments carried out by exploiting dedicated experiments suggested by the model simulations.

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PS14-25: An integrated metabolism, growth and cycle model for *Saccharomyces cerevisiae*: Validation against chemostat dataFederico Papa^{1,2}, Pasquale Palumbo^{1,2}, Marco Vanoni^{1,3}, Meike Wortel⁴, Bas Teusink⁴, Lilia Alberghina^{1,3}¹SYSBIO, Centre of Systems Biology, Milan, Italy; ²IASI "A. Ruberti", National Research Council (CNR), Rome, Italy;³Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy; ⁴Systems Bioinformatics, Amsterdam Institute for Molecules, Medicines and Systems, Vrije University Amsterdam, the Netherlands

The usefulness of a mathematical model addressing complex biological functions is given by its ability to provide quantitative agreement with a variety of experimental parameters and data, often dispersed in the literature, so to clearly define the underlying design principles governing the non-linear dynamics of the functions under investigation [1]. In this paper we formulate an integrated model providing a coarse grain representation of the main functions (metabolism, growth and cell cycle) of the budding yeast *Saccharomyces*

cerevisiae growing in a chemostat culture, suitably tuning the model parameters in order to reproduce available experimental data on metabolism, growth and cycle of *S. cerevisiae* cells growing in chemostat. The Metabolism, Growth and Cycle Model (MGC) arises from the integration of two sub-modules: the Metabolism and Growth Model (MGM) and the Growth and Cycle Model (GCM). We exploited the MGM as a *parameter generator* for some of the GCM parameters: the idea is that different environments stimulate different metabolisms, thus providing different growth conditions that, in turns, provide different cycle domains. A different set of experimental data is also exploited to verify the goodness of the model predictions.

[1] Gatenby R. (2012) *Nature*, 491, S55.

PS14-26: Non-Annotated genetic factors associated with cell wall-related processes, important in K2 susceptibility

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To preserve their surroundings from competitors numerous yeast species secrete a wide spectrum of lethal proteins, named killer toxins, aiming at the inhibition or destruction of intrusive yeast. Toxins attack various molecular targets on sensitive cells. Established mechanisms include disruption of ionic potential across the membrane, the interruption of cell division by blocking DNA synthesis, cell cycle arrest in G1, inhibition of β-1,3-glucan synthesis, and destruction of tRNA molecules by an endonuclease. Many cellular factors play important roles in modulation of the response to particular toxin. By performing high-throughput genome-wide screen we identified 332 cellular factors affecting susceptibility to *Saccharomyces cerevisiae* K2 toxin. Genes, whose deletion causes increased resistance, are directly connected to cell wall structure/biogenesis and mitochondrial function; hypersensitive mutants are deficient of genes known to be involved in osmosensory and cell wall stress signaling, ion and pH homeostasis maintenance. Among single-gene deletion mutants possessing altered resistance to K2 toxin, large group were functionally not characterized in *Saccharomyces Genome Database* (SGD) (44 ORFs) or even called as “dubious ORFs” (27), indicating uncertainty for them to be true genes. In this study, we investigated possible functional and structural interactions between non-annotated gene products, important for K2 susceptibility, and proteins, involved in cell wall structure and biogenesis. We build a protein-protein interaction network and tested K2 toxin binding efficiency to deficient in particular ORF yeast mutant. The comparison of computational analysis and experimental data allowed assigning previously uncharacterized ORFs to be functionally related to the cell wall processes and important for K2 toxin receptors formation.

PS14-27: *In silico* analysis of transcriptional regulation of F-box encoding gene SAF1 of *S. cerevisiae* during heam deficiency stress and pterostillbene treatment

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F-box encoding gene of *S. cerevisiae* *SAF1* has been shown to involve in the degradation of Aah1p in SCF dependent ubiquitination manner during entry of cells into quiescence stage. There are four transcriptional regulators *BUR6*, *MED6*, *SPT10* and *SUA7* of the *SAF1* gene entered into the SGD database. Pterostilbene is a naturally occurring phenolic compound which exhibits several important pharmacological properties including anticancer. Here we have studied the expression profiling database (GEO) of *S. cerevisiae* cells during heam deficiency stress for the expression status of the *SAF1*, AAH1 and their correlation with the global transcription factors using YeastRACT(<http://www.yeastRACT.com/index.php>) which showed the Rlm1p as novel transcriptional regulator of both the gene *AAH1* and *SAF1*. It was observed that upon heam deficiency both *AAH1* and *SAF1* expression were down regulated in comparison to control along with the listed transcriptional regulators of the *SAF1* gene. Surprisingly it was further observed that Pterostilbene treatment of *S. cerevisiae* cell showed the similar pattern of expression of *SAF1*, AAH1 and listed transcription factors during heam deficiency stress. Based on analysis of *in-silico* data, we hypothesize that pterostilbene treatment might induce the quiescent phenotype in the *S. cerevisiae* and could be helpful in studying the transcriptional regulation of *AAH1* and *SAF1* gene.

PS14-28: Control of the transcriptional repressor Mig1 in the glucose repression pathway

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The yeast AMPK/SNF1 signalling pathway controls energy homeostasis and is best known for its role in glucose derepression. Upon glucose limitation, upstream kinases phosphorylate and activate Snf1, which in turn phosphorylates and inactivates the Mig1 transcriptional repressor resulting in Mig1 cytoplasmic import and glucose repression. Under glucose repletion, Snf1 is inactivated. Mig1 becomes dephosphorylated, accumulates in the nucleus and represses a large number of genes. However, the exact mechanism and control of Mig1 dephosphorylation is still unclear. To study and analyse the behaviour of Mig1 in yeast *Saccharomyces cerevisiae* we employed Western Blotting, RT-qPCR, high gain and speed super-resolution microscopy as well as mathematical modelling and simulations. Our results suggest that expression of the MIG1 is glucose- and auto-regulated. It further appears that Mig1 shuttles between the cytosol and the nucleus under all conditions and hence its phosphorylation or dephosphorylation may occur in either compartment. Glucose regulation of the Mig1 phosphorylation state is mediated by the Snf1 kinase while dephosphorylation may be constitutive and mediated by a presently unknown protein phosphatase(s).

PS14-29: Development of an efficient glycerol utilizing *Saccharomyces cerevisiae* platform strain via adaptive laboratory evolution

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With increasing interest in biosustainable technologies, the need for converting available non-saccharide carbon sources most efficiently is emerging. Highly abundant crude glycerol, a major waste residue that is generated during biodiesel production, has attracted attention as a cheap carbon source for microbial fermentation processes. The challenge is to identify robust microbial hosts that can efficiently convert glycerol into value added products in a manner that meets the demands of industrial biotechnology. The most commonly known microbial cell factory, the yeast *Saccharomyces cerevisiae*, has been extensively applied for the production of a wide range of scientifically and industrially relevant products using saccharides (mainly glucose) as carbon source. However, it was shown that popular wild-type laboratory yeast strains (in particular CEN.PK series), extensively applied in metabolic engineering studies, did not grow or only grow very slowly in glycerol medium. In this study, a laboratory evolution approach to obtain *S. cerevisiae* strains with an improved ability to grow on glycerol was applied. An array of evolved strains, which exhibited a significant increase in the specific growth rate and a higher glycerol consumption rate, were isolated. The best performing strains were further analyzed by classical genetics and whole genome re-sequencing in order to understand the molecular basis of glycerol catabolism in yeast. The knowledge acquired in this work can be further applied for rational *S. cerevisiae* strain improvement for using glycerol as a carbon source in industrial biotechnology processes. This work is a part of the DeYeastLibrary consortium financed by ERA-IB DeYeastLibrary - Designer yeast strain library optimized for metabolic engineering applications <http://www.era-ib.net/deyeast-library>.

PS14-30: α -Ketoglutarate causes a large transcriptional reprogramming and positively affects yeast growth

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Saccharomyces cerevisiae reacts to the quality and quantity of nitrogen (N) source by controlling its intracellular uptake and by fine-tuning anabolic and catabolic processes. *S. cerevisiae* is able to use different compounds as sole nitrogen source. These sources are divided qualitatively in preferred and non-preferred. The two essential criteria underlying this classification are the growth rate supported when they are present as a sole nitrogen source and the ability of the preferred N-sources to repress processes required for the utilization of non-preferred

nitrogen sources. Amino acids used as preferred nitrogen sources yield carbon skeletons that can readily be integrated in metabolism. Glutamate is among these N-sources, since, in addition to supporting a good growth rate, generates by deamination α -ketoglutarate (α KG), one of the intermediates of the Krebs cycle. Comparison of *S. cerevisiae* grown in minimal medium with glucose 2% as carbon source and glutamate 75 mM as N-source to a reference condition, in which the source of nitrogen is 75 mM ammonium, we observed a phenotype that we named *enhanced growth* characterized by larger cell volume and average protein and RNA content during exponential growth, and a larger biomass accumulation in stationary. We then wanted to test whether the *enhanced growth* phenotype could be mimicked by providing cells with glutamate constituents, i.e. α KG 75 mM to cells plus ammonium 75 mM. During exponential growth, strain the strain GRF18 growing in this nutritional condition shows an intermediate phenotype in terms of cell size, RNA and protein content, accompanied by a significant reduction in the mass duplication time. At the end of growth GRF18 accumulates biomass comparable to cells grown in glutamate. To examine the effect of these three different nutritional condition on gene expression profiling, a transcriptomic experiment was carried out using the 'Affymetrix GeneChip ®' technology. This comprises a factorial design, in which each condition has been sampled in exponential phase, phase of glucose exhaustion and stationary phase. We will present this genome-wide transcriptional analysis and discuss the results in a systems biology perspective.

PS14-31: The number of SCB elements in the promoters of G1-regulon genes affects their expression during the G1/S phase transition

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In *Saccharomyces cerevisiae* the entrance into S-phase requires a burst of transcription of about 200 genes (G1-regulon) [1], mediated by the transcription factors SBF and MBF. They are heterodimers composed of a common regulatory subunit, Swi6, and two DNA-binding proteins, Swi4 in SBF and Mbp1 in MBF. SBF complex regulates transcription of genes involved in bud emergence and spindle pole body duplication, among which *CLN1*, *CLN2* and *PCL1*, whereas MBF complex targets genes involved in DNA replication, repair and metabolism such as *CLB5*, *CLB6* and *RNR1* [2]. By analyzing multiple published gene expression data sets, a correlation between the number of SBF binding sites in the promoters of G1-genes and their maximal expression levels have been suggested. Then, in order to better investigate this phenomenon, we used a reporter gene (*pCYC1-LacZ*), under the control of one or four SCB elements (*SWI4/6*-dependent cell cycle box), and studied its cell-cycle regulated expression by quantitative Real-Time PCR. Our results show that the higher is the number of SCB elements in the promoter, the higher is the expression level of *LacZ* gene during the G1/S transition. This is both due to an earlier activation of the transcription and to a stronger accumulation of the corresponding mRNA in the cell. To account for the experimental transcription dependence on the SCB numbers, a stochastic hybrid model which correlates the probability of activation of the main molecular players expressed by the G1-regulon (i.e. *CLN1*, *CLN2*, *CLB5*, *CLB6* and *NRM1*) with the corresponding number of SCB elements present on their promoter will be shown.

[1] Spellman et al., (1998) *Mol Biol Cell*. 9, 3273-97; [2] Mendenhall and Hodge, (1998) *Microbiol Mol Biol Rev*. 62, 1191-243.

PS14-32: Unidirectional transport of the mitochondrial GTP/GDP carrier in *Saccharomyces cerevisiae*

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Ggc1p is a yeast mitochondrial carrier protein involved in the GTP/GDP transport. This protein encoded by YDL198c gene has been shown to be a multicopy suppressor (by an unknown mechanism) of the ability of the abf2 null mutant to grow at 37°C on glycerol. The ABF2 gene whose product is involved in mitochondrial

genome maintenance in *S. cerevisiae*. Abf2 Δ cells loose mtDNA at a high rate when grown in glucose medium and show a temperature-sensitive defect on non-fermentable carbon sources. The physiological role of Ggc1p in *S. cerevisiae* is the GTP transport into mitochondria, in exchange for intramitochondrially generated GDP. In addition, ggc1 Δ cells exhibit lower levels of GTP and increased levels of GDP in their mitochondria; they are unable to grow on nonfermentable substrates, and they loose mtDNA. In the mitochondrial matrix, GTP is required as an energy source for protein synthesis; as a substrate for the synthesis of tRNA, mRNA, rRNA, and RNA primers; and as a phosphate group donor for the activity of GTP-AMP phosphotransferase and G proteins. In several organisms, GTP is synthesized in the mitochondria by succinyl-CoA ligase, which catalyzes the conversion of succinyl-CoA to succinate with the generation of GTP, and by nucleoside diphosphate kinase, which catalyzes the transfer of the phosphate from ATP to a nucleoside diphosphate, to yield nucleotide triphosphates. In *S. cerevisiae*, however, succinyl-CoA ligase produces ATP instead of GTP, and the mitochondrial nucleoside diphosphate kinase is localized in the intermembrane space and it is absent in the matrix. These observations imply that in *S. cerevisiae* GTP has to be imported into the mitochondria probably via a carrier system embedded in the inner mitochondrial membrane. Here, this protein has been overexpressed in *E. coli*, reconstituted into phospholipid vesicles, and tested for a variety of potential substrates. When citrate is present, the carrier changes the transport activity, from an antiport mechanism to an uniport mechanism. A similar response has also been observed for the protein in the mitochondria. We conclude that uniport transport of GTP is involved in the homeostasis of guanine nucleotide pool in the mitochondrial matrix.

Poster Session 15: New tools in yeast research

PS15-1: Construction of a yeast strain collection for exploration of gene function using β-estradiol-regulated gene expression

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The genetic tractability of *Saccharomyces cerevisiae* enables the construction of genome-wide mutant collections for systems-level studies. We recently described optimal promoter designs for engineering β-estradiol-responsive gene expression [1,2]. In this system, addition of β-estradiol induces nuclear transport of an artificial transcription factor, leading to binding to a synthetic promoter region and subsequent expression of a target gene. Because the artificial transcription factor is sequestered in the cytosol in the absence of β-estradiol, target genes are not activated until β-estradiol is introduced, enabling dynamic perturbation of gene expression under diverse physiological conditions. Our goal is to construct a genome-wide collection of strains carrying β-estradiol-inducible genes. To ensure that the β-estradiol-inducible gene expression system is optimal for a large-scale strain construction effort, we performed several tests. First, a yeast strain with a synthetic β-estradiol-responsive promoter driving *LEU2* grew in SD medium lacking leucine only in the presence of β-estradiol. Second, strains carrying promoter-replacement alleles of two essential genes, *ACT1* and *CDC24*, were able to grow in the presence of β-estradiol but not in its absence. Third, when the expression of *TPS2* was made conditional on β-estradiol, the strain did not grow at 37°C, as expected for a strain lacking the *TPS2* gene product. Addition of β-estradiol restored wild-type-like growth. Finally, overexpression of *GLN3* is known to cause a growth defect. A strain containing an inducible allele of *GLN3* arrested its growth in the presence of β-estradiol. To assess the relationship between inducer concentration and the level of target gene activation, we created a genomically integrated β-estradiol-inducible allele of GFP. GFP signal increased in a dose- and time-dependent manner after β-estradiol addition. Following removal of β-estradiol from the media, GFP signal decreased to near-background levels within 24 hours. A collection of strains in which every gene can be induced by β-estradiol (one at a time), with single-gene precision, will be a useful addition to the yeast functional genomics toolbox. With validated constructs in place, we are now in a position to engineer such a collection.

[1] McIsaac et al. (2014) *Nucleic Acids Research* 42, e48; [2] McIsaac et al. (2013) *Nucleic Acids Research* 41, e57.

PS15-2: Heat shock impact of recombinant protein secretion

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Expression of complex heterologous proteins such as viral glycoproteins and secreted human proteins in yeast is often limited by several bottlenecks in the secretory pathway of yeast *Saccharomyces cerevisiae*. It was shown earlier that synthesis of measles virus hemagglutinin (MeH) is inefficient mostly due to a bottleneck in the translocation of viral protein precursors into the endoplasmic reticulum (ER) of yeast cells. Our studies in the Institute of Biotechnology (Vilnius University) found that heat shock with subsequent induction of MeH expression at 37°C improved translocation of MeH precursors to about 3-fold when applied at higher cell densities in late-log glucose or ethanol growth phases. We also investigated whether heat shock has a positive effect in other steps of secretory pathway and whether these conditions can improve the expression of other heterologous proteins in yeast cells. No positive effect of heat shock was found on the expression of MeH and Influenza neuraminidase glycoproteins fused with α-factor signal sequence. Expression of viral nucleocapsid proteins Measles N, Mumps NP, hPIV1-N and hPIV3-N in heat-shocked yeast cells was increased by 1.3, 1.2, 1.5 and 1.2 fold, respectively. The same temperature conditions increased both secretion titer and yield of another heterologous protein human GRP78/BiP by about 50%. Heat shock conditions increased the secretion of native sequence human calreticulin to 1.2 fold, but no increase of secreted human interferon fused with yeast

secretion signal sequences was observed. Furthermore, heat shock at the late-log glucose growth phase also improved endogenous invertase yield by approximately 2.7-fold. These results suggest that heat shock treatment is a promising approach for improvement of heterologous protein expression in yeast, however it's yet to be determined when this strategy is useful and when it has no positive impact on heterologous protein expression in yeast.

PS15-3: Innovative visualization tools to interconnect multiple data types at SGD

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The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a comprehensive resource of curated molecular and genetic information on the genes and proteins of *Saccharomyces cerevisiae*. The increasing use of genome-wide technologies to explore transcriptomes, proteomes, and epigenomes has created the need to analyze the roles of sequences and other elements across an entire genome/proteome. Efficient, effective organization, search, identification, and analysis of similar experiments and results across varied organisms requires the collection of standardized metadata and development of cutting edge, novel visualizations that will summarize and highlight the complexity and connectedness of the data. To avoid the common pitfall of trying to visualize too much data at once, with “hairball” network diagrams or a multitude of tracks, SGD is developing visualization tools that layer or filter data akin to a faceted or elastic search. For example, the interaction network graph at SGD displays interactions based on the evidence (number of experiments) supporting each interaction using an adjustable slider, which can be tuned to be more or less restrictive based on user preference. When data cannot be summarized in a decipherable network diagram they are condensed and/or summarized. For example, phenotype annotations are summarized based on the mutant type (e.g., null, overexpression) or strain background. SGD has recently developed a tool called the “Variant Viewer” to facilitate the visualization of sequence variation, such as SNPs, between different strains of *S. cerevisiae*.

PS15-4: Metabolic engineering of *Saccharomyces cerevisiae* for production of chemicals

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The chemical industry is one of the world's largest with a turnover of 4 trillion US dollars annually, accounting for 4.3% of global Gross Domestic Product (GDP). World production of chemicals is growing at higher rate than world population due to increasing consumption. Most of the chemicals are derived from fossil sources, which is not sustainable on a long-term. The change towards bio-based chemicals produced by fermentation of renewable feedstocks requires robust high-performing strains. The development of such strains has been greatly facilitated by the new enabling synthetic biology and systems biology tools. I will present how synthetic biology tools, based on CRISPR-Cas9, RNA interference, and biosensors, are used to engineer laboratory and industrial *S. cerevisiae* strains for production of chemicals. I will also illustrate the importance of adaptive laboratory evolution for generating tolerance phenotypes and deciphering tolerance mechanisms. Application examples that will be presented include 3-hydroxypropionic acid, a building block for acrylate-based products (diapers, acrylic paints, acrylic polymers, etc.), and aromatic compounds with nutraceutical properties. In summary, I will show how modern synthetic biology and metabolic engineering tools can be used to achieve production of non-inherent chemicals in yeast at high titer, rate and yield.

[1] Jensen et al. (2014) *FEMS Yeast Res* 14, 238-248; [2] Borodina & Nielsen (2014) *Biotechnol J* 9, 609-620; [3] Kildegaard et al (2014) *Metab Eng* 26C, 57-66; [4] Borodina et al (2015) *Metab Eng* 27, 57-64; [5] Stovicek et al (2015) *Metab Eng Commun* 2, 13–22; [6] Li & Borodina (2014) *FEMS Yeast Res* doi: 10.1111/1567-1364.12213.

PS15-5: PTR-ToF-MS and bioprocesses: Potential in monitoring VOCs release by eukaryotic microbes

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The release of volatile organic compounds (VOCs) by eukaryotic microbes is of interest for several fields, comprising food, environmental, biotechnological and medical applications. In addition, it represents an intriguingly opportunity to conceive and to confirm new hypotheses in fundamental biology and in breeding sciences. For example, VOCs studies in an -omics perspective are generally defined as volatome, indicating with this terms the organic volatile subset of metabolome. The various techniques for VOC analysis generally aim to combine either sample throughput or analytical insight. From this point of view, Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-ToF-MS) represents a valid compromise, with the advantages of on-line process monitoring and non-invasive analysis. In order to maximize the advantages of on-line bioprocess monitoring, we coupled PTR-ToF-MS with an auto-sampler, adding a tailored data analysis tools. We demonstrated the applicability of our comprehensive methodology (automatic sampling, PTR-ToF-MS analysis and tailored data handling and analysis) the study *Saccharomyces cerevisiae* volatile organic compounds released during alcoholic fermentations. In particular, considering bread-making bioprocess, we use this approach i) to differentiate bakery yeast starter cultures in reason of their release of VOCs and to analyze the effect on VOCs productivity as a function of ii) different bakery yeast starter cultures/flour combinations, iii) the interaction between *S. cerevisiae* and *Lactobacillus sanfranciscensis* as model microorganisms in the sourdough environment, iv) different commercial aromatic yeast starter cultures for bakery.

PS15-6: Systems level understanding of cell polarity regulation

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All cells - including those in our body - possess some degree of asymmetry or ‘polarity’, which is key to their healthy function and if disrupted can lead to serious cellular malfunctions like those found in cancer. We have reconstructed with unprecedented spatiotemporal resolution the molecular networks that regulate cell polarity using an interdisciplinary strategy - combining genetics, microscopy and computational approaches - and focusing on the polarity machinery of the archetypal model organism *Schizosaccharomyces pombe* (fission yeast). Using network analysis methods, we have identified the core set of genes/proteins that regulate cell polarity in fission yeast and obtained a basic interaction ‘network’ map connecting those genes/proteins, as well as discovered new molecular links between cell polarity, cell cycle and cytokinesis control. We determined the detailed network topology and the functional hierarchy among polarity regulators in this yeast species. Based on these and other earlier results we built a mathematical model that captures the polarity pattern changes throughout the cell cycle of fission yeast cells.

PS15-7: Characterization of stress free imaging conditions for long-term multi-color time-lapse microscopy**Andreas P. Cuny, Gregor W. Schmidt, Fabian Rudolf***ETH Zurich, Department of Biosystems Science and Engineering, Basel, Switzerland*

S. cerevisiae and *S. pombe* are suitable to investigate dynamic processes at single cell resolution using live-cell fluorescence microscopy. The enabling technologies for these assays are: automated microscopes, a range of fluorescent proteins cassettes for endogenous tagging, long-term culturing conditions relying on agar pads or microfluidic devices and software for cell recognition and tracking. Fluorescent proteins spanning the whole visible spectrum of light are available. That allows the visualization of one or more proteins of interest in the same cell. However, little attention has been devoted to the phototoxic effects caused by fluorescence excitation light. We aimed to identify imaging conditions which do not exert a stress on the cells during long-term imaging. We monitored the growth rate of yeast cells, to quantify the cellular stress imposed by imaging. Cells were cultured in a microfluidic chip and exposed to different doses of excitation light in a time-lapse imaging experiment. From the images the cell division times were extracted and plotted against the light dose. The excitation wavelengths were chosen based on the brightest available fluorophores in each part of the spectrum. Additionally, the applied light doses correspond to imaging conditions suitable for the observation of endogenously tagged proteins. We observed no phototoxicity for wavelengths between 520nm and 620nm for all light intensities tested. We did observe an increase in cell cycle times in *S. cerevisiae* and *S. pombe* upon exposure with light between 380nm to 480nm. For light in the range of 420nm to 480nm phototoxicity could be diminished by lowering the light dose. However, fluorophores with excitation spectra spanning this range should be used for tagging of abundant proteins that are sufficiently visible at low light doses. For light in the range of 380nm to 400nm an increase in cell cycle duration was observed for all light intensities, implying that non-invasive fluorescence imaging using light in that range appears impractical. Taken together, fluorescent proteins excited with light in the range of 520nm to 620nm are preferable when detection of less abundant proteins, like transcription factors, requires maximal sensitivity. We believe that our work helps researchers to choose optimal combinations of fluorescent proteins and time-lapse microscopy setups to allow stress free observation of cellular processes.

PS15-8: Construction and characterization of a yeast strain designed for mercury bioremediation**Jessica P. Fuentes Rivera, Ana Clara G. Schenberg, Elisabete J. Vicente***Research Center for Biotechnology, University of São Paulo, São Paulo, SP, Brazil*

A promising candidate for mercury bioremediation is the bacterial metalloregulatory MerR protein, the molecule showing the highest affinity and selectivity for Hg²⁺ ions known so far. This work describes the construction of a recombinant strain of *Saccharomyces cerevisiae* expressing a bacterial MerR protein on the cell wall, upon use of the yeast Flo1 protein C-terminal region as an anchor. The merR gene was PCR amplified from *Cupriavidus metallidurans* CH34 total DNA and the amplified fragment was inserted in the pGEMT-Flo428 plasmid, resulting in the pGEMT-MF plasmid, which contains the "merR-Flo428" gene fusion. This gene fusion was then cloned downstream of the yeast α -factor signal peptide coding sequence (SS) in the pPIC9K plasmid, giving rise to the pPIC9KMF plasmid. The resulting "SS-merR-Flo428" DNA fragment was removed from this plasmid and inserted in the *S. cerevisiae* expression vector pMA91, under control of the *S. cerevisiae* 3-phosphoglycerate kinase (PGK) promoter, to obtain the pMA91MF plasmid. This plasmid carrying the MerR anchoring/expression cassette was used to transform the *S. cerevisiae* YPH252 strain and the transformant clones were selected by genetic complementation of the leu2 mutation. The cell surface display of MerR in the recombinant strain YPH252/pMA91MF was confirmed by transmission electron microscopy (TEM). Mercury resistance was increased in the recombinant strain YPH252/pMA91MF, as the maximum survival to HgCl₂ increased from 90 μ M to 120 μ M. After 10 min of incubation in 5.0 uM HgCl₂, the recombinant strain showed an Hg²⁺ binding capacity 54% higher relative to the YPH252/pMA91 control strain. After 10 min of incubation in 10.0 μ M HgCl₂, the recombinant strain showed an Hg²⁺ binding capacity 84% higher than the control strain. However, the binding rate to Hg²⁺ decreased upon increasing external ion concentration and reached saturation after 120 min of incubation in 15.0 uM HgCl₂. The rapid initial adsorption of Hg²⁺ suggests an instantaneous binding of

Hg₂₊ to the MerR molecules anchored on the cell surface of the recombinant yeast strain. These results confirm the functionality of the MerR surface display in yeast, which increased significantly the mercury biosorption capacity of the cells, thus presenting potential to be used in the bioremediation of waters contaminated with mercury.

PS15-9: A Shuttle vector series for precise genetic engineering of *Saccharomyces cerevisiae*

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Shuttle vectors allow for an efficient transfer of recombinant DNA into yeast cells and are widely used in all fields of yeast research. While available shuttle vectors are applicable in many experimental settings, their use in quantitative biology is hampered by an insufficient copy number control. Moreover, they often have practical constraints, like limited modularity and few unique restriction sites. We constructed the pRG shuttle vector series consisting of single-copy and multi-copy integrative, centromeric, and episomal plasmids. The plasmids contain *S. cerevisiae* or heterologous marker genes for selection in all commonly used auxotrophic yeast strains, like W303, CEN.PK, and designer deletion strains. The vectors feature a modular design and a large number of unique restriction sites, enabling an efficient exchange of every vector part and adjustment of the plasmids for specific needs. The plasmids integrate into the host genome via a scarless double-crossover recombination mechanism resulting in stable single-copy integration. An intermediate copy number is obtained by multiplying genes cloned in the MCS prior to integration. More than 30 plasmid copies can be integrated by targeting the abundant Ty1 delta elements in the yeast genome. As centromeric and episomal plasmids give rise to a heterogeneous cell population, we comprehensively analyzed their copy number distribution and loss behavior. For centromeric plasmids, we observed that the majority of cells contained two or more plasmid copies. This copy number distribution resulted from frequent asymmetric plasmid segregation during cell division. Also plasmid-free cells were generated predominantly via this mechanism. Overall, our shuttle vector series supports the efficient cloning of genes and their maintenance in yeast cells with a predictable copy number.

PS15-10: Curating multi-allele phenotypes in pombase

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PomBase, the model organism database for fission yeast, makes the comprehensive and detailed representation of phenotypes one of its key features. We have made considerable progress in developing a modular ontology, the Fission Yeast Phenotype Ontology (FYPO), for phenotype descriptions, and in making phenotype annotations for single mutants available. Canto, the PomBase community curation tool, provides an intuitive interface for curators and community users alike to associate alleles with FYPO terms and supporting metadata such as evidence, experimental conditions, and annotation extensions that capture expressivity and penetrance. The PomBase web site displays phenotype annotations on gene pages, and supports FYPO term searching by name or ID. We are now extending the PomBase phenotype annotation resources to annotate phenotypes observed in double mutants, triple mutants, etc. The Chado database underlying PomBase supports annotation of specific alleles, singly or in combinations, by associating phenotypes with genotypes which in turn link to their constituent alleles. Extensive additions and adaptations of the Canto phenotype annotation interface enable curators and researchers to capture multi-allele phenotype data and metadata. We invite comments on extending the PomBase gene page display and search options to accommodate and use the new data.

PS15-11: A Novel approach to simultaneously tracking two species of single fluorescent molecules in 3D with high temporal resolution using astigmatic microscopy and its application to tracking Snf1/Mig1 signal transduction in *Saccharomyces cerevisiae*

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Visualising single molecule interactions in living cells in real-time presents several challenges for microscopy. The limited photon budget of fluorescent proteins and the low signal to noise ratio even with high gain EMCCD cameras when run at the short exposure times needed to resolve diffusive movement *in vivo* necessitates the use of high intensity excitation light. The approach presented here utilizes a set of techniques and parameters chosen to allow selective imaging and tracking in three dimensions with good axial precision of the fluorescent proteins eGFP and mCherry for up to 0.5 s before bleaching at 4.4 ms exposure time and 200 frames per second alternating frame laser excitation. The Snf1 protein kinase of *Saccharomyces cerevisiae* plays an important role in its adaptation to glucose limitation. The main mechanism is through the Snf1/Mig1 pathway. By using yeast strains with genetically integrated eGFP and mCherry tags on several components in the pathway, the described system can simultaneously track the location of singular Mig1 and another tagged pathway agent.

[1] Lutfiyya et al. (1998) *Genetics* 150: 1377–1391; [2] Hedbacher, Carlson (2008) *Frontiers in Bioscience* 13, 2408-2420; [3] Ahuatzi et al. (2007) *Journal of Biological Chemistry* 282, 4485-4493; [4] Bo Huang et al. (2008) *Science* 319, 810-813.

PS15-12: Synthetic biology tools for fast and reliable strain engineering in the oleaginous yeast *Yarrowia lipolytica*

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The production of chemicals in engineered microorganisms can be of advantage over chemical synthesis e.g. in terms of biosustainability and stereoselectivity. Oleochemicals, derived from plant and animal fats, have a wide range of applications, such as transportation fuels, cosmetic and feed ingredients. Oleochemicals can also be produced by microbes from sugars, wastestreams and other feedstocks. Oleaginous yeasts accumulate up to 90% of their cellular dry weight as lipids and are therefore an excellent host for the production of the above mentioned chemicals. One of the most applied oleaginous yeast species is *Yarrowia lipolytica*, which is generally regarded as safe. The construction of high-producing cell factories involves a various amount of genome edits as e.g. integration of several (heterologous) genes, the down-regulation or deletion of native genes. As genome editing tools for *Y. lipolytica* are limited, we seek to develop a genome editing toolbox, which enables fast, flexible and reliable strain engineering. We will present our efforts to adopt the CRISPR-Cas9 system, which allows specific genome editing of multiple genome regions simultaneously, for *Y. lipolytica*. CRISPR-Cas9 introduces a double strand break in a specific genome region and can be repaired by homologous recombination using an expression construct or knockout construct as repair template. The system does not require any selection markers as only cells with a repaired double strand break can survive. The system will allow knock-out and knock-ins of DNA fragments. For knock-ins we are testing a range of insertion sites, which we identified as being highly transcribed. We will illustrate the applicability of the genetic toolbox by engineering *Y. lipolytica* for production of an oleochemical of choice.

PS15-13: Studying yeast volatolome with Proton-Transfer-Reaction Time-of-Flight Mass
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Yeast metabolism plays a key role in the production of flavor compounds in wine thus affecting its final quality and sensory profile. Volatile compound concentration is influenced by the growth characteristics of yeast strains. For this reason a rapid and non-invasive screening of the yeast volatolome is of outmost relevance. Proton-

transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) showed promising results in monitoring *Saccharomyces cerevisiae* volatile production in dough and bread [1]. Sixteen different wine yeast strains of *Saccharomyces paradoxus*, *Saccharomyces cerevisiae* and their hybrids were selected for studying volatile organic compound (VOCs) release by a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) coupled to a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany). The samples (five biological replicates of each yeast strain, the substrate used for their growth (solid YPD, Yeast Peptone Dextrose) and lab air) were left for 3 days in dark at 30°C. Then, the headspace of each sample was automatically measured 5 times every 260 minutes for 60 seconds which guaranteed total replacement of headspace by pure air. Due to high ethanol production during yeast growth, an inert gas dilution was applied in order to prevent primary ion depletion and formation of ethanol clusters which might affect the quantification of volatiles. Data processing of PTR-ToF-MS spectra consists of dead time correction, external calibration and peak extraction [2]. One-way ANOVA (p-value < 0.01) for each yeast strain higher than the substrate reduced the dataset from 349 to 114 mass peaks. Principal component analysis of total yeast aroma profiles showed a clear separation of yeast strains. In addition, we were able to identify significant differences in off-flavor sulfuric compounds produced in higher amount by wild strains (both *S. cerevisiae* and *S. paradoxus*). Sulfur-containing VOCs produced during the yeast metabolism reactions are known to play significant role in wine aroma adding both fruity notes and off-flavor [3]. In this work, for the first time, PTR-ToF-MS coupled to a multipurpose headspace automated sampler was applied for a rapid and non-invasive analysis of the yeast colonies. The technique was successful in characterizing of different yeast strains and identifying difference in the release of important classes of compounds.

[1] Makhoul S, Romano A, et al. (2014) *J Mass Spectrom* 49, 850-859; [2] Cappellin L, Biasioli F, et al. (2010) *Int J Mass spectrom* 290, 60-63; [3] Swiegers J. H., Pretorius I. S. (2007) *J Appl Microbiol Biotechnol*. 74, 954-60.

PS15-14: ATP Analog-Sensitive Pat1 protein kinase as a tool for induction of synchronous fission yeast meiosis at physiological temperature

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Synchronous cultures are often indispensable for studying meiosis, especially for physical analyses of DNA and proteins. Here we present an ATP analog-sensitive allele of Pat1 protein kinase developed for induction of synchronous meiosis in the fission yeast *Schizosaccharomyces pombe* at physiological temperature. Chemical inactivation of an ATP analog-sensitive form of the Pat1 kinase (Pat1(L95A), designated *pat1-as2*) by adding the ATP analog 1-NM-PP1 in G₁-arrested cells allows the induction of synchronous meiosis at optimal temperature (25°C). Our studies showed that this allele eliminates detrimental effects of elevated temperature (34°C), which is required to inactivate the commonly used temperature-sensitive Pat1 kinase allele (*pat1-114*). The addition of the *mat-Pc* gene to a *mat1-M* strain further improves chromosome segregation and spore viability. Comparison of transcriptional program of *pat1-as2* and *pat1-114* mutants during meiosis demonstrated that some transcripts are spliced in early (*pat1-as2 mat Pc*) and some in middle (*pat1-114, pat1-as2*) stage of meiosis. We analyzed the timing of splicing of *rem1* and *mde3* meiotic genes. We observed that pattern of splicing depends on temperature. This indicates that the expression patterns are significantly affected by higher temperature used for inactivation of *pat1-114* compare to *pat1-as2*. Our plan is to further improve system of *pat1-as*-induced synchronous meiosis at optimal temperature.

PS15-15: Reverse Two Hybrid Assay (RYTHA), a systematic approach for identifying genes and pathways that regulate a specific protein-protein interaction**Ifat Lev, Marina Volpe, Shay Ben-Aroya***Bar-Ilan University, 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 Assay" (RYTHA). 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 (YTH). 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.

PS15-16: PomBase community literature curation**Antonia Lock¹, Midori A. Harris², Kim Rutherford², Jürg Bähler¹, Stephen G. Oliver², Valerie Wood²**

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PomBase obtains its highest-quality data by manual curation of the fission yeast literature, which provides experimentally supported annotations representing gene structure, function, expression and more. Approximately 5000 papers suitable for manual curation published on the model organism *Schizosaccharomyces pombe* to date, of which about 2100 have been fully curated. To supplement the work of its small staff of professional curators, PomBase has developed a community curation model that enables fission yeast researchers to participate directly in curating data from new publications. As of April 2015, the community has contributed annotations for over 260 *S. pombe* publications. Community curation improves the visibility of recent publications, and enables researchers and professional curators to work together to ensure that PomBase presents comprehensive, up-to-date and accurate representation published fission yeast experiments. Furthermore, because PomBase is one of only three databases that provide manual literature curation for fungal species, electronic data transfer of high-confidence *S. pombe* annotations to other fungal species is an essential source of function-related data for the latter. Community contributions to PomBase therefore support research not only within the fission yeast community, but also throughout the broader community studying all fungi.

PS15-17: Versatile tools for multicolor-protein labeling and live-cell imaging in yeast cells**Ivana Malcová¹, Marian Farkasovsky², Lenka Senohrabková¹, Jiří Hasek¹**

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Analyses of proteins behavior and localization in living yeast cells are performed in many laboratories over the world and various tools were developed enabling C-terminal tagging of genes/proteins of interest either on plasmids or in the genome. Our sets of integrative cassettes for C-terminal tagging with fluorescent proteins by homologous recombination are based on vectors constructed by Janke *et al.* [1]. Each set is comprised of three plasmids with different selection markers – G418/kanMX4, nourseothricin/cloNAT/natNT2 and hygromycin /hphNT1. One set of vectors for PCR-based preparation of integrative cassettes carries the mCherry fluorescent protein from *Discosoma* sp (pFM571, pFM689, pFM699), the second set contains a yeast variant of

the photostable red fluorescent protein from *Entacmaea quadricolor* TagRFP-T (pIM572, pIM690, pIM700) and the third set carries the yeast enhanced mTagBFP (pIM573, pIM691, pIM701). It is possible to amplify by PCR all the integrative cassettes with one set of primers designed for a specific gene. Moreover, these primers were chosen in such a way that the 3' reverse primer is possible to use also for amplification of pFa-derived deletion cassettes as well as of those employing the cre/lox system [2-3]. Thus one need to re-design the 5' forward primer only and various combinations of deletions and/or fluorescently tagged genes of interest can be easily prepared in one cell. Plasmids for the C-terminal tagging with the photostable TagRFP-T protein (pIM23 and pIM35) are essentially pUG23 and pUG35 (Gueldener and Hegemann, unpublished) where GFP was exchanged for TagRFP-T by the Gibson assembly method. The photostable variant of the red protein is very useful when multiple images are necessary to be taken in time without losing fluorescence intensity. The commercially available GFP-tagged yeast strains collection and our cassettes with two types of red and one blue fluorescent protein thus enable an easy preparation of strains with multicolor-labelled proteins and their consecutive analyses not only by live cell microscopy but also by biochemical techniques since antibodies exist for all three fluorescent proteins. Supported by grants CSF P305/12/0480, GACU 1180213, VEGA Grant 2/0002/15.

[1] Janke et al., (2004) Yeast, 21:947-962; [2] Carter and Delneri, (2010) Yeast, 27, 765-775; [3] Gueldener et al., (2002) Nucl. Acids. Res. 30: e23.

PS15-18: CRISPR/Cas9: a molecular swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*

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A variety of techniques for strain engineering in *Saccharomyces cerevisiae* have recently been developed. However, especially when multiple genetic manipulations are required, strain construction is still a time-consuming process. This study describes new CRISPR/Cas9-based approaches for easy, fast strain construction in yeast and explores their potential for simultaneous introduction of multiple genetic modifications. An open-source tool (<http://yeastriction.tnw.tudelft.nl>) is presented for identification of suitable Cas9 target sites in *S. cerevisiae* strains. A transformation strategy, using *in vivo* assembly of a guideRNA plasmid and subsequent genetic modification, was successfully implemented with high accuracies. An alternative strategy, using *in vitro* assembled plasmids containing 2 gRNAs was used to simultaneously introduce up to 6 genetic modifications in a single transformation step with high efficiencies. Where previous studies mainly focused on the use of CRISPR/Cas9 for gene inactivation, we demonstrate the versatility of CRISPR/Cas9-based engineering of yeast by achieving simultaneous integration of a multi-gene construct combined with gene deletion and the simultaneous introduction of 2 single-nucleotide mutations at different loci. Sets of standardized plasmids, as well as the web-based Yeastriction target-sequence identifier and primer-design tool, are made available to the yeast research community to facilitate fast, standardized and efficient application of the CRISPR/Cas9 system.

PS15-19: Growing together: From a single cell to the self-organized 2D multicellular assembly

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A population of microorganisms like *Escherichia coli* or *Saccharomyces cerevisiae* is not a simple sum of all of its individuals but rather it is a complex structure with many intricate interactions which produce a coupled spatiotemporal variability. It is an interdependent multicellular community composed of different local microenvironments with specific growth rates as well as production and uptake of nutrients, wastes and other molecules. There are many environmental and genetic determinants that give rise to complex colony morphology and internal dynamics such as intercellular communication, cell surface properties, cell-cell adhesion strength, sensing nutrients level, secretion of extracellular matrix, etc. [1]. Such multicellular communities exhibit various adaptive benefits like more efficient proliferation, access to resources and niches that require a critical mass and cannot effectively be utilized by isolated single cells, collective defense against antagonists that usually eliminate

isolated single cells, and optimization of population survival when confronted with diverse physical, chemical, nutritional or biological challenges [2]. Although there is an obvious contrast between homogeneous environments on one side and heterogeneous environments on the other, until recently most of research has been focused either on single cells or on bulk liquid cultures, putting aside the complexity of multicellular communities – the most encountered microorganism formation in nature. Several teams use microfluidic systems to study microorganisms in a complex chemical landscapes either on single cell level or on population level. However, they rarely study the local structure of cell assemblies at the single cell level which is the aim of this project. To this date I developed an experimental microfluidic platform in which a monolayer of yeast cells is trapped inside a cell chamber which facilitates the use of time-lapse microscopy and analysis at a single cell level. Diffusion and uptake of nutrients (e.g. glucose) by cells forms a gradient of nutrients inside the cell chamber. I quantified the growth rates of cells in glucose gradients formed by a range of different starting glucose concentrations as a function of their position inside the chamber, i.e. as a function of their distance from the glucose source. I looked at gene expression of hexose transporters and glucose sensors which I will further expand with key genes in glucose metabolism, diauxic shift and stress response. Furthermore, I observed the galactose metabolism regulation in different ratios of glucose to galactose concentrations. Finally, I used agent based modelling software to preliminary model the growth of a monolayer of cells in a gradient of nutrients inside a cell chamber.

[1] Granek J.A., Magwene P.M. (2010) *Plos Genetics* 6, e1000823; [2] Shapiro J.A. (1998) *Annu. Rev. Microbiol.* 52, 81-104.

PS15-20: Dynamic quantitative metabolomics for comparative studies in yeast

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Compared to genomics or transcriptomics, the analysis of intra- and extracellular metabolites under certain conditions is a relatively new research field. The development of standard sample generation procedures and reproducible measurement techniques in the last years made it possible to generate reliable data which for the first time could prove or disprove common hypotheses and textbook knowledge. Due to the instant reaction of the metabolome to every change in the surrounding of the biological system under investigation, quantitative metabolomics is one of the most powerful phenotyping tools, but at the same time one of the less robust ones. By high frequent sampling, we could show that the metabolome of *Saccharomyces cerevisiae* is dynamic within all stages of a batch cultivation. This illustrates how strong the result of an analysis is dependent from the right choice of sampling point and cultivation system. Samples for comparative metabolome studies in yeast and other microorganisms are most commonly generated during the exponential growth phase, assuming that the metabolome remains in a steady-state as long as the cells are dividing with maximum speed. Our data proves, that this assumption only holds true within narrow limits. In a comparative study we analysed time resolved metabolome data of more than 100 yeast segregants, generated from the cross between S296 and YJM789. The observed intra- and extracellular dynamics enable the differentiation between single strains that appeared to be metabolically identical on the basis of common single point measurements. Apart from the rich data set, the project provides a feasible experimental protocol for the generation of dynamic intra- and extracellular metabolome data for high strain numbers.

PS15-21: Discovering novel ubiquitin variant inhibitors of Deubiquitinases *in vivo*: strategies using budding yeast

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Deubiquitinating enzymes (DUBs) are key regulators of the ubiquitin pathway that catalyze the removal of ubiquitin from target substrates. Hence, these enzymes affect a wide variety of cellular processes by influencing the localization, stability and function of their targets. Importantly, the inhibition of DUB activity has emerged as a promising therapeutic strategy for several diseases, including cancer and neurodegenerative disorders. Despite

this, a paucity of specific and potent inhibitors aimed at DUB pathways has hindered attempts to exploit them for therapeutic benefit. To address this issue, ubiquitin can be exploited as a scaffold to generate highly specific and potent inhibitors of DUBs, called ubiquitin variants (UbV) [1]. Using phage-display we screened complex libraries comprising $\sim 7.5 \times 10^{10}$ unique UbVs to identify specific and potent DUB inhibitors¹. Although effective, an inability to purify many disease-relevant DUBs has limited the applicability of *in vitro* screening systems and thus *In vivo* screening techniques would circumvent this issue. In a proof-of-principle study we have established the GAL4 based yeast two-hybrid (Y2H) system as a powerful platform for detecting interactions between UbVs and DUBs. To establish & optimize a Y2H screening pipeline, we developed targeted UbV libraries against the DUB USP2. We have identified numerous UbV binders of USP2 that are currently being assessed. Furthermore, we are adapting this system to allow for the high-throughput screening of massively diverse and combinatorial UbV libraries. In conjunction with established screening methods, we will use this platform to discover novel inhibitors against disease-relevant DUBs. This work will make 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.

[1] Ernst, A. et al. (2013). *Science*. 339(6119), 590–5.

PS15-22: CRISPR-PCS: An efficient and versatile chromosome splitting technology in yeast

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Recent studies have revealed that most of the industrially useful traits such as stress tolerance are controlled by multiple genes. For that reason, development of novel technologies that can manipulate large genomic region or multiple genomic loci is strongly required. In the budding yeast *Saccharomyces cerevisiae*, we developed a novel chromosome engineering technology, called PCR-mediated chromosome splitting (PCS). PCS technology enables splitting of a yeast chromosome at any desired genomic locus and generating two functional chromosomes. However, owing to the low splitting efficiency, splitting event is limited to only one site splitting in PCS, which prevents high-through put chromosome manipulation such as simultaneous multiple splitting and marker-free splitting. Because chromosome splitting in PCS technology is based on the mechanism of homologous recombination, enhancement of homologous recombination activity is the key to enhance chromosome splitting efficiency. It is well known that double strand break (DSB) greatly enhances the homologous recombination activity by activating the DSB repair pathway in *S. cerevisiae*. Recently, a novel genome engineering technology called CRISPR/Cas, which can induce site-specific DSB induction, is developed. CRISPR/Cas system is also shown to work in yeast. Based on these previous findings, we hypothesized that induction of DSB by CRISPR/Cas system prior to splitting would strongly increase splitting efficiency in PCS technology. In this study, we developed a highly efficient and versatile chromosome engineering technology called CRISPR-PCS. CRISPR-PCS can split yeast chromosomes with much higher efficiency as compared to conventional PCS without any chromosomal rearrangements, thereby it enables multiple chromosome splitting (even a quadruple splitting is possible) simultaneously and marker-free chromosome splitting. In addition, CRISPR-PCS can enhance the efficiency of chromosome deletion. In conclusion, CRISPR-PCS will be a powerful tool not only for breeding of useful yeast strains but also for elucidation of genomic function by creating a yeast cell with various chromosome constitutions.

PS15-23: Versatile and user-friendly microfluidics for high-resolution long-term imaging of yeast

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Long-term culturing and analysis of growing cell populations at single-cell resolution are key technologies to advance our knowledge on cellular variability, cellular aging processes or the regulation of growth in different environmental conditions. However, due to the exponential growth of yeast populations, long-term culturing necessitates continuous removal of excess cells – a non-trivial task. Microfluidic technology can be used to ensure controlled culture conditions including removal of excess cells to obtain time-resolved data of live yeast cells over extended time periods. Unfortunately, the use of microfluidic technology is often restrained through

the necessity to have specialized microfabrication equipment (e.g. plasma bonding machines), complex setups (e.g. high number of chip-to-world connections) or their elaborate handling and low reliability (e.g. long setup times and frequent failure). We developed a convenient microfluidic platform that enables researchers to carry out long-term culturing of *S. cerevisiae* or *S. pombe*. Setting up the chip and cell loading takes one hour. Loading of cells and chip assembly is accomplished in a single step by use of a standard laboratory pipette and a dedicated vacuum channel. The chip has a minimum number of fluidic connections, only one inlet and one outlet per medium condition. The chip can be easily fabricated by PDMS replica molding from a master wafer. No plasma activation procedure is required for chip assembly, which allows re-use of the microfluidic system and further reduces cost and fabrication effort. We show that *S. cerevisiae* and *S. pombe* can be cultured inside the chip for at least three days. Up to twelve yeast strains can be investigated under up to four different media conditions in parallel. For each yeast strain parallel investigation of thousands of cells is possible, while excess cells are continuously removed, which ensures long-term operation of the system. We characterized our microfluidic culture system with respect to the availability of oxygen and nutrients for the cells. We show that oxygen does not become limiting inside the microfluidic chip when cells are grown aerobically with ethanol as the carbon source. Furthermore, we show that nutrient availability does not limit cell growth, by measuring the cell cycle times of *S. cerevisiae* inside the microfluidic chip. We believe that our platform can be widely applied to analyzing yeast cells and other unicellular organisms.

PS15-24: A composite pan-genome of *Saccharomyces cerevisiae* using AGAPE (Automated Genome Analysis PipelinE)

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Despite the recent release of a hundred *Saccharomyces cerevisiae* strain sequences, connecting population genome evolution with functional and phenotype variation in yeast remains challenging. This is due in part to the limitation of current comparative analyses, which rely on solely a reference genome defined from a single strain. Variation in genes that are not present in the reference genome is often overlooked, and cannot be examined with this method. Attempts have been made to overcome this problem in prokaryotes using pan-genome analysis, which aggregates sequence data from multiple strains to define a full set of genes within a species. However, these analyses have remained problematic for eukaryotic genomes due to their increasingly complex gene structures. We have developed a eukaryote pan-genome analysis pipeline for *S. cerevisiae*, and have made it freely accessible online. The pipeline includes steps for assembly, annotation, and variation-calling, and identifies novel genes that are not present in the S288C reference. Using the pipeline to analyze 120 *S. cerevisiae* strains, we have generated a composite pan-genome of *S. cerevisiae*. All genes can be assigned into core (always present), dispensable (sometimes present), and unique (rarely present) genomes. We investigate two aspects of variation within the pan-genome: (1) variation in the core genome, (2) patterns of absence or presence of genes in the dispensable and unique genomes. We have summarized this genetic variation with a phylogenetic tree based on Single Nucleotide Polymorphisms (SNPs) in the core genome, and with another phylogenetic tree based on clustering of gene presence/absence patterns in the dispensable and unique genomes. We will also present results from an analysis of population structure based on SNPs in the core *S. cerevisiae* genome.

PS15-25: Development of New expression platform for anticancer arginine-degrading enzymes in the methylotrophic yeast *Hansenula polymorpha*

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Malignant cells often exhibit specific metabolic defects such as impaired anabolism of amino acids and are concomitantly hypersensitive to artificial deprivation of these amino acids. This feature is exploited in anticancer enzymotherapies based on single amino acid, e.g. asparagine or arginine, deprivation. To achieve efficient arginine depletion *in vivo*, two recombinant enzymes, bacterial arginine deiminase and human arginase I have been successfully exploited in clinical trials. Both enzymes were heterologously expressed in several bacterial hosts. Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyzes the conversion of L-arginine to L-ornithine

and urea. We utilized the methylotrophic yeast *Hansenula polymorpha*, one of the well known efficient eukaryotic expression hosts, for construction of new original producers of secretory recombinant human arginase I (rhARG). By i) creating the strains that harbor multiple copies of rhARG expression cassette under alternative promoters using auxotrophic selectable markers; ii) designing optimal media conditions; iii) applying efficient protocol for purification of HIS6-tagged rhARG, we achieved high yield of rhARG (appr. 15,000 IU (up to 15 mg) from 1 liter of culture). Efficiency of purified preparations of rhARG as inhibitors of tumor growth have been proven in laboratory tumor models in 2D and 3D cultures. To overcome the limitations of rhARG non-optimal pH range, recently the substitution of Mn²⁺ for Co²⁺ in ARG1 active center, that lowers pH optimum and also stabilizes enzyme at physiological pH, was reported. Introduction of certain point mutations into the second-shell metal ligands of rhARG led to a remarkable optimization of its characteristics for *in vivo* use [1]. Our preliminary data indicate that it is possible to express secretory cobalt-containing rhARG directly from the genetically modified yeast producers. Evaluation of yeast-produced rhARG as a therapeutic agent is in progress.

[1] Stone et al. (2012) *Journal of Controlled Release* 158, 171-179.

PS15-26: Non-conventional yeast *Hansenula polymorpha* as a tool for green synthesis of the new bionanomaterials

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Recent advances in nanotechnology have enabled the exploration of nanomaterials for diverse applications. Metallic nanoparticles (NPs) are important object of nanotechnology due to their potential utilization in industry and medicine. Metallic NPs can be synthesized through different methods, namely, using spark discharging, electrochemical and chemical reduction and cryochemical synthesis. Synthesis of NPs using microorganisms as an emerging field of bionanotechnology has received considerable attention due to a growing need of environmentally friendly technologies in materials synthesis. Furthermore, green synthesis of metallic NPs offer better manipulation, stabilization and control over crystal growth due to slower kinetics. These green synthetic processes are rapid, cost-effective, and they can easily be scaled up. Many living organisms (plants, bacteria, fungi, yeasts), polysaccharides, DNA, RNA, proteins, and polypeptides are known to produce nanostructured mineral crystals and metallic NPs with properties similar to chemically synthesized materials. Biological agents secrete a great deal of enzymes that are able for enzymatic reduction of metallic ions. The exact mechanism for the synthesis of NPs using biological agents has not yet been elucidated, but it has been suggested that various biomolecules are responsible for the synthesis of NPs. In this research, the recombinant strain of thermotolerant yeast *Hansenula polymorpha* was used for "green" synthesis of different types of metallic NPs. The process of metallic ions reduction followed by bioNPs formation *in vivo* (by the yeast culture) and *in vitro* (in extracellular liquid after cells removing) were studied. The methods of transmission electron microscopy, scanning electron microscopy, fluorescent electron microscopy, UV-visible absorption spectroscopy were used to confirm the reduction of Ag⁺, Au³⁺, Cd²⁺, Pt⁴⁺, Pd³⁺ and other ions to corresponding monometallic or bi-metallic NPs. Influence of various parameters, namely: temperature, pH, time, concentration of corresponding salts on metal NPs biosynthesis was studied. Our findings might be eventually interesting for the fundamental and applied biological sciences, namely for the study of molecular mechanisms of cell protection from stress, caused particularly by exposure to toxic compounds and for the development of methods for the synthesis of nano-sized fluorescent labels for clinical diagnostics and testing.

PS15-27: TheCellMap.org: storing and visualizing genetic interactions in *S. cerevisiae*

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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.

PS15-28: Yeast as a model in aerospace research: simulating spaceflight environment on earth

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Weightlessness is a challenging environment in which organisms experience several stresses, including oxidative damage, which is possibly due to changes in oxygen/intermediary metabolism or to dysfunction in the oxidative stress response. In order to investigate the effects of weightlessness on yeast as a model organism - with a specific focus on glutathione homeostasis - we developed a novel microgravity simulator system in which the weightlessness conditions are obtained by a suitable medium and a flow-lift suspension. In details, in this bioreactor the gravitational vector acting on cells is counterbalanced by the hydrodynamic thrusts created by a bottom-up spiral flow of a fluid having increased density. Here we present the validation of the proposed bioreactor: *i.* by modeling its efficiency using the main physical parameters as relative velocity, shear stress, and oxygen transport; *ii.* by comparing the experimental results obtained on Earth in *Saccharomyces cerevisiae* using the proposed bioreactor, with those obtained in space during the SCORE (*Saccharomyces cerevisiae* Oxidative Stress Response Evaluation) mission onboard the FOTON-M3 spacecraft.

PS15-29: Integrating genome-wide datasets into *Saccharomyces* Genome Database

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The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a comprehensive resource of curated molecular and genetic information on the genes and proteins of *Saccharomyces cerevisiae*. The emergence of large-scale, genome-wide technologies has widened the scope of functional annotation beyond that of individual genes to entire genomes, allowing us to identify shared and divergent features between genomes. We have collected published data from whole-genome studies that employ a diverse set of modern techniques, including tiling arrays, cDNA clone libraries, TIF-seq, single and paired end RNA-seq, and serial analysis of gene expression (SAGE). These divergent methodologies target different genomic regions, such as ncRNA, transcription start sites (TSS), transcripts, poly(A) sites, and antisense RNA. Using ontologies and controlled vocabularies, metadata were curated from more than 1500 datasets from NCBI's GEO repository (Gene Expression Omnibus). These data will be available for straightforward querying at SGD via a faceted search tool to facilitate user access to yeast genomic data.

Poster Session 16: Yeast comparative and evolutionary biology**Poster Session 16: Yeast comparative and evolutionary biology****PS16-1: The Kti11 and Kti13 proteins form a heterodimer with a dual role in elongator-dependent tRNA modifications and diphthamide modification of eukaryotic elongation factor2****Karin D. Breunig¹, Rene Zabel¹, Sebastian Glatt², Christoph W. Müller²**¹Martin-Luther-Universität Halle-Wittenberg, Institut für Biologie, Weinbergweg 10, D-06120 Halle (Saale), Germany; ²European Molecular Biology Laboratory, Structural and Computational Biology Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany

The yeast proteins Kti11 alias Dph3 and Kti13 have been identified as potential regulators of the tRNA modification function of the Elongator complex. Here we demonstrate that Kti13 is a large WD40 protein, which heterodimerizes with the small zinc and iron-binding Kti11 protein. We present the crystal structures of Kti13 alone and in complex with Kti11 and confirm interacting residues through mutational analysis *in vitro* and *in vivo* [1]. Kti11 binds Kti13 through conserved residues at the top of the Kti13 β-propeller. The ability of both proteins to heterodimerization is not only required for modifications of tRNA but also for diphthamide modification of translation elongation factor eEF2 via the DPH complex. Hence two steps in translation, tRNA binding to the ribosome and translocation of the bound tRNAs are affected. By complementation analysis of the *kti11 kti13* double mutant we show that *Arabidopsis thaliana* Kti11 and Kti13 homologs together can functionally replace the yeast proteins. Hence, the role of the Kti11/Kti13 heterodimer is likely to be conserved in plants and the role of plant Elongator in tRNA modification [2] involves homologs of Elongator associated proteins identified in yeast.

[1] Glatt S., Zabel R. et al. (2015) *Structure* 23, 149-160; [2] Mehlgarten C., Jablonowski D. et al. (2010) *Mol Microbiol* 76, 1082-1094.

PS16-2: Comparative genomics of an evolved industrial *Saccharomyces cerevisiae* strain reveals cell periphery-related proteins as major genomic targets during the adaptation to combined heat and hydrolysate stress**Daniel P. Brink¹, Valeria Wallace-Salinas¹, Dag Ahrén², Marie-Francoise Gorwa-Grauslund¹**¹Applied Microbiology, Department of Chemistry, Lund University, Lund, Sweden; ²Microbial Ecology Group, Department of Biology, Lund University, Lund, Sweden

Adaptive laboratory evolution (ALE) is an important complement to rational metabolic engineering for improving the tolerance of yeast strains for bioethanol production. Previously we have reported on the evolved industrial *Saccharomyces cerevisiae* strain ISO12 that has acquired improved ability to grow and ferment in the presence of lignocellulose-derived inhibitors at high temperature (39°C) [1]. In the present study, comparative genomics of ISO12 and the parental strain Ethanol Red (ER) was used to uncover the molecular mechanisms behind the novel phenotype of ISO12. Reads from Illumina whole-genome sequencing of ER and ISO12 were used to call sequence variants (relative to the S288c reference strain) in order to identify strain-unique SNPs and INDELs in the parental and the adapted strain. It was found that ORFs with non-synonymous variants were enriched in cell periphery-related Gene Ontology terms and these results were further supported by subsequent *in silico* analyses. These included calculation of the non-synonymous to synonymous substitution ratio (K_a/K_s) with the aim to find genes that were subject to positive selection, and modelling of the Copy Number Variations (CNVs) between the strains. Finally, the genomes of the two strains were *de novo* assembled in order to assess the CNVs in genomic material that was not present in the reference strain. The genomic characterisation of ISO12 was found to match physiological and lipidomic results that were obtained in parallel, with a convergence towards alterations to peripheral lipids and proteins as an evolutionary strategy to withstand the combined stress of heat and hydrolysate. This study also demonstrated that the long-term molecular effects of adaptation to these stressors had a polygenic basis.

[1] Wallace-Salinas V, Gorwa-Grauslund MF (2013) *Biotechnol Biofuels* 6, 151.

PS16-3: Genomic and transcriptomic landscapes within a protoploid yeast species

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Exploration of genetic diversity and gene expression variations between isolates of the same species are both essential to obtain an overview of the evolution of genomes and regulatory networks, which underlie phenotypic diversity. Numerous studies have characterized intraspecific variations and focused on a large number of individuals using *Saccharomyces cerevisiae* as a model organism. While extremely important, this wealth of data stands in contrast to our limited knowledge in other yeast species. In this perspective, we sought to have a view of the genomic and transcriptomic landscapes in the protoploid species (i.e. which diverged from the *S. cerevisiae* lineage prior to its ancestral whole genome duplication): *Lachancea kluyveri* (formerly *Saccharomyces kluyveri*). We first performed a population genomic analysis on a large number of isolates. Our results clearly showed that distinct recombination and substitution regimes coexist and lead to different evolutionary patterns. More precisely, we revealed that a 1-Mb region on the chromosome C is a relic of an introgression event and is characterized by a higher GC content, a higher sequence diversity as well as a large set of unique unannotated genes. In this context, we then explored the transcriptomic landscape within this collection of isolates by RNA-seq. Interestingly, our comparative transcriptomic analysis clearly showed a link between gene evolutionary history and expression behavior. Indeed, genes recently acquired (such as genes present in the introgressed region) or under function relaxation tend to be less transcribed, show a higher intraspecific variation and are less involved in network. Moreover, utilizing this approach in *L. kluyveri* also highlighted specific regulatory network signatures in aerobic respiration, amino-acid biosynthesis and glycosylation, presumably due to its different lifestyle. Our datasets shed an important light on the genome evolution in yeast and its impact on transcription.

PS16-4: Functional analysis of the NADP-Dependent glutamate dehydrogenase (NADP-KlGDH1) of *Kluyveromyces lactis* and (NADP-LkGDH1) of *Lachancea kluyveri* VS paralogous genes GDH1/GDH3 of *Saccharomyces cerevisiae*

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Gene redundancy is a common characteristic of living organisms, which may occur for a single gene, chromosomal segment or whole genome. Gene duplication may be a source for genetic material useful to develop new or specialized functions. The genome of the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), arose from a whole genome duplication event (WGD), after which, a selective loss and a subsequent retention of a group genes, allowed the development of a facultative metabolism. The paralogous genes *GDH1* and *GHD3* codify for glutamate dehydrogenases; and these enzymes form hexamers and are implicated in the glutamate biosynthesis. Between these two paralogous enzymes there is 87% of identity in amino acids sequence. Previous studies in our laboratory have shown that Gdh1 and Gdh3 display different kinetic properties. Gdh1 has a higher affinity for α -ketoglutarate than Gdh3. *GDH1* is expressed in glucose as the unique carbon source while *GHD3* is repressed under this condition and its expression is derepressed when glucose is exhausted or in ethanol as the only one carbon source. *K. lactis* and *L. kluyveri*, diverged from the *Saccharomyces* lineage before the WGD event and have a unique orthologous NADP-Gdh (*KlGdh1* o *LkGdh1*). When Gdh1 is compared with *KlGdh1* and *LkGdh1*, an 80% identity in amino acids sequence is observed, and Gdh3 a 74% of identity with *KlGdh1* and *LkGdh1*. The aim of this project is to characterize the *KlGdh1* enzyme and *LkGdh1* enzyme following three different approaches: i) Phenotypic analysis of the single mutants *Klgdh1Δ*, *Klglt1Δ*, *Lkgdh1Δ*, *Lkglt1Δ* and double mutants *Klgdh1Δ-Klglt1Δ* and *Lkgdh1Δ-Lkglt1Δ* grown under different physiological conditions ii) kinetic characterization of the enzymes and, iii) analysis of the expression profiles. So far, we have shown that the single mutants *Klgdh1Δ* and *Lkgdh1Δ* are a glutamate braditrophs and lack NADP-Gdh activity, and double *Klgdh1Δ-Klglt1Δ*, *Lkgdh1Δ -Lkglt1Δ* mutants are glutamate auxotrophs. Analysis of the kinetic properties of enzymes is under way.

Poster Session 16: Yeast comparative and evolutionary biology**PS16-5: Using engineered populations to study the genetic basis of drug resistance in *Saccharomyces cerevisiae***

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We aim to use the tools of quantitative genetics to tractably model complex causal relationships between genotype and phenotype, specifically ATP Binding Cassette (ABC) transporter mediated drug resistance in *Saccharomyces cerevisiae*. ABC transporters carry out a variety of cellular functions, are associated with multiple drug resistance, are conserved in organisms ranging from prokaryotes to humans, and are well studied mechanistically and genetically. To this end, we have back-crossed a yeast strain with 16 ATP Binding Cassette transporters deleted (ABC-16) to a pool of barcoded wild-type strains. We have assembled a collection of 6,845 genotyped and barcoded haploid segregants of this cross, thus engineering a cell population that is readily assayed for strain-specific competitive growth under a variety of conditions by high-throughput barcode sequencing. Initial growth data in 16 different antifungal and anticancer drugs shows biologically reproducible complex genetic interactions of three or more genes. We have reproducibly predicted and verified, for example, that strains with a quadruple deletion of the ABC transporters SNQ2, YBT1, YCF1, and YOR1 are surprisingly resistant to the antifungal drugs fluconazole and ketoconazole, and that this effect depends upon the presence of PDR5.

PS16-6: Comparative genomics of natural fertile hybrids of *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces paradoxus*

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Natural inter-species hybrids of the genus *Saccharomyces* have been documented, and in some cases the hybridization gave origin to commercially useful strains. The most renowned example is that of *Saccharomyces pastorianus*, generated by the mating of *S. cerevisiae* and *S. eubayanus*, and widely used for pale ale beer production [1]. Other natural interspecies hybrids have been more rarely documented, probably because the low viability of their offspring strongly impact on the survival of the strain type when environmental changes are detrimental. While natural *S. cerevisiae* × *S. bayanus* hybrids have been found, hybrids of *S. cerevisiae* and *S. paradoxus* are much more rare, as indicated by the few documented isolations [2], possibly because both the different habitat and the fact that their progeny is usually not fertile. We isolated a *S. cerevisiae* × *S. bayanus* hybrid and a *S. cerevisiae* × *S. paradoxus* hybrid from the intestine of the hornet *Vespa crabro* giving spores showing 100% viability and we fully sequenced the genome of each of the four meiotic segregants after sporulation. Using available complete and draft genome sequences of *S. cerevisiae*, *S. bayanus* and *S. paradoxus* as reference, we tested whether the two parental genomes are still physically separated after the mating by comparative genomics analysis.

[1] Libkind D, Hittinger CT, et al. (2011) *Proc Natl Acad Sci U S A*; 108(35):14539-44; [2] Lucia Morales and Bernard Dujon (2012) *Mol Biol Rev*. 76(4): 721-739.

PS16-7: Variation in transcription factor binding sites and open chromatin profiles in yeast strains from different environments

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Differences in transcription factor (TF) binding profiles have been shown to exist between different yeast strains, although such studies have to date been limited to just one or two factors. Such differences may result in divergent sets of genes activated by a given TF, and may have arisen as adaptation to selective pressures during long-term growth in differing environments. Binding profile differences can arise from divergences in cis-type effects (e.g., changes to binding motifs at regulated genes), the trans-acting TFs themselves (e.g., TF binds to novel motif), cis- and/or trans-type effects in accessory binding proteins, or from any combination of these. We have Myc-tagged 50 TFs in 5 ecologically diverse haploid *S. cerevisiae* strains and have performed ChIP-Seq analysis to detect TF binding profile differences among them. Additionally, we have performed ATAC-seq [1] on all 5 strains to visualize regions with “open chromatin”, and have furthermore performed RNA-seq on the strains. We thus can make multidimensional correlations between TF binding, chromatin status, and transcription levels. All 5 strains have had their genomes fully sequenced, allowing us to discern possible divergences due to cis- or trans-type effects and to additionally detect TF binding patterns, open chromatin sites, and transcription patterns in novel genomic regions that do not exist in laboratory strains. Our tagged TFs include those involved in response to carbon/nutrients, stress response, filamentous growth, response to pH, and several other pathways. Together our results provide insights into the role that evolution in differing environments has played in the control of gene regulation.

[1] Buenrostro et al. (2013) Nature Meth 10, 1213-8.

PS16-8: Spatio-temporal variations of alpine soil microbial communities - influences of plants, soil chemistry and biogeographic history

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Fungi are key actors of important ecosystem services such as recycling of soil organic matter, plant productivity or carbon sequestration, which led to active research to better understand the links between composition of soil fungal communities and biotic or abiotic factors of the environment. Alpine habitats are a suitable model to perform these studies for several reasons. First, there display a minimal human disturbance. Second, at short scales (meter to landscape) there is a high contrast of abiotic (soil chemistry, temperature) and biotic (plant species composition) environment, and there is no limitation of fungal species dispersion. Finally, at large scale it is possible to find similar habitats, which allows accounting for differences in dispersion and in biogeographic history. During the last ten years, we have performed field surveys from the plot to the multi-regional scale on alpine habitats using a beta diversity approach: to determine the differences of fungal communities between different habitats. The fungal communities were characterised by molecular fingerprinting or massif sequencing. These studies showed that soils from neighbouring, but contrasted alpine habitats exhibit a highly different fungal composition, and that this composition exhibits a clear seasonality. At the landscape level, the distribution of fungal communities is specific of each plant community, and is related to two proxies of plant productivity: annual radiations and soil organic matter. Studies on two grasslands encompassing three biogeographic regions revealed the endemism of Fungi, but highlighted fungal biotrophic species (pathogenic and endophytic Fungi) constitute a core mycobiome of each grassland and may be closely associated to the dominant plant. Finally, studies greenhouse experiments of alpine plants and in field surveys of the alpine cushion plant *Silene acaulis* revealed that plant species and plant genotype are correlated with species composition of soil fungal communities; this correlation is well established for biotrophic taxa. Taken together, these results suggest that plant species or genotype are involved in the selection of fungal biotrophic strains, and that soil chemical and climatic characteristics are involved on the selection of saprophytic Fungi.

Poster Session 16: Yeast comparative and evolutionary biology**PS16-9: The segregants of *Debaryomyces hansenii* killer yeast**Monika Grzegorczyk, Xymena Połomska, Barbara Żarowska, **Zbigniew Lazar**

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The aim of the study was to characterize two yeast segregants of *Debaryomyces hansenii* AII4b killer strain: S (smooth) and R (rough), recently distinguished from fed batch culture in YPG medium. The analysis concerned cell and colony morphology in various media as well as growth rate and the ability to synthesize and secrete killer toxins in fed batch cultures in YPG medium. The study showed that AII4bS exhibited both: higher biomass yield and killer toxin activity in YPG cultures in comparison to AII4bR segregant. The IGSAF analysis (intergenic spacer rDNA amplification and AluI fingerprinting) confirmed the taxonomic affiliation of both segregants to *D. hansenii* species. The research revealed that there are significant differences between AII4bS and AII4bR segregants and further, more insightful analyzes should be conducted.

PS16-10: Regulation of mating in the methylotrophic yeast *Pichia pastoris***Lina Heistinger**, Brigitte Gasser, Diethard Mattanovich

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Pichia pastoris (*Komagataella* sp.) is a methylotrophic yeast with a preferentially haploid lifestyle, being able to form diploids by mating. In contrast to *Saccharomyces cerevisiae*, where mating between cells of opposite mating-type is well understood, the mechanisms of mating and mating-type switching are by far less well characterized in *P. pastoris*. Generally, the mating-type of a yeast cell is determined by the transcription of the *MATa* and *MATalpha* genes, regulating the activity of specific genes involved in mating and sporulation. In *P. pastoris*, mating is homothallic and homologs of the *MATa* and *MATalpha* genes are found in two different positions in the genome. Both loci are on the same chromosome and are flanked by identical sequences. It has been proposed that the position of the *MAT* genes determines the mating-type and switching takes place by recombination [1]. To gain a better understanding of mating regulation in *P. pastoris*, strains with deletions of the *MAT* genes and the pheromone receptors involved in mating signaling (Ste2 and Ste3) were generated. The effects of these gene deletions on mating efficiency and transcript levels of important factors involved in mating were investigated.

[1] Hanson, S. J., Byrne, K. P., & Wolfe, K. H. (2014). *Proc. Nat. Acad. Sci. USA*, 111(45), E4851–E4858.

PS16-11: Investigation of the prevalence of antagonistic pleiotropy**Lucas Herissant¹, Dave Yuan¹, Elizabeth Jerison², Atish Agarwala¹, Daniel Fisher¹, Michael Desai², Dmitri Petrov¹, Gavin Sherlock¹**¹Stanford University, USA; ²Harvard University, USA

Pathogenicity, drug resistance and cancer progression are examples of mutation-driven processes, where increased selective advantage is conferred upon cells carrying new mutations. While these mutations may be beneficial in one specific condition, they may be deleterious in other conditions, a phenomenon known as Antagonistic Pleiotropy (AP). AP is thought to lead to evolutionary trade-offs and the persistence of deleterious alleles. But what is the prevalence of AP? Which genes or pathways are more likely to be involved in AP, and under which conditions? Is there any specific type of mutation that results in AP? One study investigated AP and observed ~14% of non-essential gene deletions display AP [1] but the prevalence and nature of AP for beneficial mutations is largely unexplored. To answer these questions, we are using an experimental system that allows us to track and measure the fitness values of 500,000 separate lineages within an evolving yeast population via DNA barcodes [2]. With this system, we are able to identify lineages that gain a beneficial mutation, based on how their frequencies increase over time. We will then select clones that harbor beneficial mutations in order to measure their fitness in alternate conditions. We will infer AP for any clones that carry beneficial mutations in the first environment that now show a fitness lower than wild-type in at least one alternate environment, and perform whole genome sequencing on clones of interest. These data will result in the largest set of fitness measurements for adaptive mutations ever collected across multiple environments. It will allow us to determine, for example, the extent of AP among new beneficial mutations and any correlations between the magnitude of the beneficial effect a mutation confers in one environment and whether it exhibits AP in other environments. The results will indicate whether beneficial mutations in certain pathways are more likely to exhibit AP and will

provide the first insight of how mutation-driven processes can confer selective evolution or why deleterious alleles can be conserved upon evolution.

[1] Qian et al. (2012) *Cell Rep.* 2, 1399–410; [2] Levy et al. (2015) *Nature* 519, 181–6

PS16-12: Species-wide survey reveals the complex landscape of the genetic origin of reproductive isolation within natural yeast populations

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Dissecting the molecular basis of reproductive isolation within a single species is valuable to understand the pattern of genetic differentiation as well as the onset of speciation. By combining classical genetic and high-throughput genomic approaches, yeasts have emerged as powerful models to get a deep insight into the mechanisms involved in reproductive isolation across natural populations. Interspecific and more recently intraspecific surveys highlighted large-scale chromosomal rearrangements as the major mechanism leading to reduced hybrid fertility on rich media. Nevertheless, evident examples of genetic incompatibilities resulting from negative epistasis remained undetected, possibly due to incomplete penetrance of antagonistic interactions under permissive laboratory conditions. Using natural populations of *Saccharomyces cerevisiae*, we analyzed here a large number of highly fertile crosses on rich media and tested their offspring viabilities on different environmental conditions. Of all instances tested, over 24% (117/481) showed loss of offspring viability to various degrees, indicating that condition specific negative epistasis were surprisingly common. Using high-resolution genomic mapping, we identified the first case of classic two loci Dobzhansky-Müller genetic incompatibility leading to offspring respiratory deficiency and demonstrated that the incompatibility was due to interaction between a nonsense mutation in a nuclear-encoding mitochondrial gene and a tRNA suppressor. We also showed that the presence of the tRNA suppressor gives rise to specific fitness effects across natural isolates and could provide transient adaptive advantages in fluctuating environments. Moreover, further evidences clearly pointed out that additional epistasis between the suppressor and multiple loci lead to increased variance of offspring fitness upon crosses. So far, we have mapped the genomic regions involved and the causal genes as well as mutations are currently under investigation. Overall, our data revealed the unforeseen multiplicity of the complex genetic origin of reproductive isolation within a single yeast species.

PS16-13: Acquisition of the ability to assimilate mannitol by *Saccharomyces cerevisiae* by spontaneous mutation in the genes encoding the general corepressor Tup1–Cyc8

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Saccharomyces cerevisiae normally cannot assimilate mannitol, a promising brown macroalgal carbon source for white biotechnology. The molecular basis of this inability remains unknown; however, previous work showed that artificial expression of *DSF1* and *HXT17*, which respectively encode a putative mannitol-2-dehydrogenase and a putative MFS transporter, confers the ability to assimilate mannitol on *S. cerevisiae* [1]. We found that cells capable of assimilating mannitol arose spontaneously from wild-type *S. cerevisiae* strain BY4742 during prolonged culture in mannitol-containing medium [2]. Based on microarray data, complementation analysis, and cell-growth data, we demonstrated that the acquired ability to assimilate mannitol was due to spontaneous mutation in the genes encoding Tup1 or Cyc8, which constitute a general corepressor complex that regulates many kinds of genes [2]. We also showed that a *S. cerevisiae* strain carrying a dysfunctional truncation allele of *CYC8* (*CYC8Δ1139–1164*) exhibited superior salt tolerance relative to other ethanologenic microorganisms; this characteristic would be highly beneficial for production of bioethanol from marine biomass. Thus, we succeeded in conferring the ability to assimilate mannitol on *S. cerevisiae* via loss of function of Tup1–Cyc8, thereby facilitating production of ethanol from mannitol. In addition, we introduced *CYC8Δ1139–1164* into the chromosome of *S. cerevisiae* strain D452-2, which had been used as a host in several fermentations from glucose, cellobiose, xylose, galactose, inulin, xylose/glucose mixture, or xylose/cellobiose mixture to ethanol, and also that from glucose to isobutanol (e.g., [3]). We also introduced *DSF1/HXT17* into BY4742 and D452-2

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via an expression plasmid, yielding four strains: (i) BY4742 CYC8Δ1139–1164, (ii) D452-2 CYC8Δ1139–1164, (iii) BY4742 DSF1/HXT17 on expression plasmid, and (iv) D452-2 DSF1/HXT17 on expression plasmid. We are now comparing the fermentation performance of these four strains, especially in regard to production of ethanol from mannitol.

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PS16-14: Quantifying separation and similarity in a *Saccharomyces cerevisiae* metapopulation

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Identifying and quantifying the population processes occurring in microbes can help our understanding of their evolutionary histories and potential future trajectories. Microbes perform essential processes vital to the functioning of the biosphere and have wide reaching impacts on global economies due to the roles they play in producing quality agricultural commodities. They are also widely used as model systems to test fundamental hypotheses about biological processes. Despite their undisputed importance to both commerce and scientific research, we have a poor understanding of microbial population biology and ecology. Here we report an in-depth quantitative analysis of population structure and migration in the budding yeast *Saccharomyces cerevisiae* to provide a more detailed account of the population processes occurring in microbes. Over 10 000 individual isolates were collected from native plants, vineyards and spontaneous ferments of fruit from six major regions spanning 1000 km across New Zealand. From these, hundreds of *S. cerevisiae* genotypes were identified and a suite of analytical methods were employed to provide a comprehensive quantitative analysis of both population structure and rates of migration. Within each geographic region no genetic differentiation was detected, even between populations inhabiting native forests and vineyards; however at distances greater than ~100 km, the New Zealand *S. cerevisiae* population exhibits varying degrees of population structure. This is complemented with estimates of bidirectional rates of migration between different geographic regions which correlate with the movement of fruit by the New Zealand wine industry. This suggests anthropogenic activities may influence microbial population patterns and diversity and in turn may influence their potential evolutionary trajectories.

PS16-15: A genetic approach for identifying factors contributing to prezygotic isolation between *Saccharomyces* species

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Understanding interspecies genetic isolation drives models of speciation – a cornerstone of evolution. An approach identifying genes that affect species isolation will have broad implications for understanding the forces that establish species boundaries and maintain species integrity. In *Saccharomyces*, interspecific hybrids are common, which makes this an informative organism to study factors that either increase or decrease genetic isolation. Previous studies have focused on post-zygotic genetic incompatibilities in these hybrids, but because *Saccharomyces* can grow indefinitely without undergoing meiosis, these do not necessarily represent a barrier to establishing a hybrid lineage. Hence, we sought to investigate prezygotic genetic isolation between species by mating every non-essential *S. cerevisiae* haploid strain in the deletion collection to *Saccharomyces uvarum* – the most distantly related species in the *sensu stricto* clade that can still mate. These interspecific matings are successful about 0.25% of the time, thus providing possibilities for significant increases or decreases in mating efficiency. Using this method we scanned the *S. cerevisiae* genome for genes important to interspecific zygote formation. We have identified two new sterility genes that were previously annotated as having an unknown function. We have identified 10 genes whose null mutants are only sterile in interspecific matings. We have also found genes that appear to hinder interspecific mating, because their null mutants are superior interspecific maters, some in a hybrid-specific way. Highly representative categories for interspecific incompatibilities of all types include genes associated with vesicle function, ribosomal proteins, and mitochondrial proteins. Mitochondrial incompatibilities have previously been shown to cause post-zygotic sterility, making this a

particularly interesting category. Vesicles and ribosomal proteins are novel molecular and cellular processes related to hybrid incompatibility. Together these genes provide the first insights into prezygotic interspecific barriers to hybridization in *Saccharomyces*, and may provide insights into broader forces that affect genetic and molecular isolation between species.

PS16-16: Genomic and Structural Comparative Analysis of Trehalose-6-Phosphate Synthase Protein

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Trehalose-6-Phosphate synthase (Tps1) is the first enzyme in trehalose biosynthetic pathway. Tps1 synthetizes trehalose-6-phosphate using glucose-6-phosphate and UDP-glucose as substrates [1,2]. Cells lacking Tps1 not only are affected in the production of the reserve carbohydrate trehalose, but also show a massive accumulation of sugar phosphates, and a drop of ATP concentrations upon glucose addition that eventually leads to lethality. We have recently demonstrated the crucial implication of Tps1 in yeast survival against different stress conditions [3]. These results underline the general importance of the protein, and/or its product T6P, as a global regulator of metabolic functions and energy homeostasis. In the present project we are carrying out a genomic and structural comparative analysis within several Tps1 homologous proteins in order to identify which ones are able to fully complement the *tps1Δ* mutation phenotypes with the purpose of determining the main domains and its implication on the global function of the protein. Selection of candidate proteins was done by multiple sequence alignment and the corresponding DNA sequences were cloned in the yeast expression vector YCplac33 under the control of the strong PGK yeast promoter. The growth of the resulting strains in different mediums reveals that as expected the bacterial homologous proteins are not able to restore the growth of the yeast in fermentable sugars, but surprisingly we observe the same phenotype for the complementation with *TPS1* from *Arabidopsis thaliana*, *Drosophila melanogaster* and the Crabtree positive *Schizosaccharomyces pombe*. Growth and viability on various carbon sources and under stress conditions assessed by flow cytometry, as well as metabolites profiles after pulses of glucose, are currently underway in order to determine which of the selected homologous proteins are able to fully complement these *S. cerevisiae tps1Δ* mutation phenotypes.

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PS16-17: Studies on functional divergence between *Saccharomyces cerevisiae ALT1* and *ALT2* using *Kluyveromyces lactis K1ALT1* and *Lachancea Kluyveri LkALT1* as ancestral type yeast

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Gene duplication has a relevant role in evolution, since diversification of paralogous genes allow the emergence of new or specialized functions from the preexisting ones. *Saccharomyces cerevisiae* experimented Whole Genome Duplication (WGD) about 100 million years ago. Comparisons between *Saccharomyces* and *Kluyveromyces* or *Lachancea* lineages suggest that the lasts lineages diverged before WGD of *Saccharomyces*. It is thus possible to consider that *Kluyveromyces* or *Lachancea* physiology is more similar to the ancestor which did not undergo through the WGD, these is why both are considered as “ancestral type yeasts”. Aminotransferases constitute an interesting model to study diversification of paralogous genes since aminotransferases constitute biosynthetic and catabolic pathways whose opposed action relies on a single catalytic site. *ALT1* and *ALT2* are two paralogous genes present in *S. cerevisiae* genome, which encode 65% identical proteins. Previous results showed that Alt1 displays alanine aminotransferase activity while Alt2 only show a reminiscence of these activity. Alt1 is localized in the mitochondria and Alt2 in the cytosol. *ALT1* is alanine-induced showing an expression profile of a gene involved in amino acid catabolism, conversely, *ALT2* expression is alanine-repressed, indicating a role in alanine biosynthesis. Since a double *alt1Δ alt2Δ* mutant is not an alanine

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auxotroph it can be concluded that there exists a yet unidentified alternative pathway for the alanine biosynthesis. *K. lactis* and *L. kluyveri* only have one ALT1-ALT2 ortholog: KIALT1 and LkALT1 respectively. Through the characterization of the null mutant of the orthologous genes, we discovered that KIALT1 and LkALT1 are the principal pathways of alanine catabolism, in each one of these yeasts, although in both cases an alternative alanine biosynthetic and catabolic pathway is present, although it has not been identified yet. Kinetic analysis of KIALT1 and LkALT1 shows that both display alanine aminotransferase activity, and are localized in the mitochondria, but while LkALT1 is alanine-induced KIALT1 is not, suggesting that the biosynthetic and catabolic capacity displayed by alanine aminotransferases has been delegated to Alt1. The characterization of KIALT1 and LkALT1 and their encoded products will allow the proposition of a model for the divergence of *ALT1* and *ALT2*.

PS16-18: Tolerance towards lignocellulosic inhibitors at low pH in *Saccharomyces cerevisiae* for 2nd generation bioethanol production

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Several challenges need to be tackled to reach a bio sustainable ethanolic fermentation from lignocellulose hydrolysates by the yeast *Saccharomyces cerevisiae* [1]. Among them, the combined tolerance towards different kinds of inhibitors formed during pre-treatment including weak acids (acetic acid and formic acid), furaldehydes (2-furaldehyde and 5-hydroxymethyl furaldehyde) and phenolics (Vanillin and p-hydroxy benzoic acid) is of particular importance. In fact, this property should not only be obtained but also maintained at low pH in order to prevent bacterial contamination [2]. The objectives of the present work were to understand how individual compounds contributed to the inhibition pattern in a mixture of weak acids, furaldehydes and phenolics as well as to identify and implement adaptation strategies to increase the cellular inhibitor detoxification thereby achieving similar growth and fermentation conditions at low pH with a combination of inhibitors.

[1] Almeida J.R., Runquist D. et al. (2011) *Biotechnology journal*. 6, 286-99; [2] Sàncchez i Nogué V., Narayanan V. and Gorwa-Grauslund M. (2013) *Appl Microb and Biotech*. 97, 7517-25.

PS16-19: Application of generalized linear model to reveal nonessential genes of deletion mutants with significant morphological phenotypes beyond natural yeast strains

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Phenotypes are variable within species, with high phenotypic variation in the fitness and cell morphology of natural yeast strains due to genetic variation. A gene deletion collection of yeast laboratory strains also contains phenotypic variations, demonstrating the involvement of each gene and its specific function. However, to date, no study has compared the phenotypic variations between natural strains and gene deletion mutants in yeast. If the variations generated by the set of individual gene deletion mutants are smaller than those of the natural variation, this suggests that the most distinct morphologies among the natural strains are due to their genetic complexity. Otherwise, if the variations of the set of gene deletion mutants are larger, then that deleted gene beyond the natural strains is possibly functional in the natural strains and important for the maintenance of natural yeast morphology. In this study, we compared variation of phenotype between 110 most distinct gene deletion strains and 36 typical natural yeast strains by 501 morphological traits. The 501 morphological traits were quantified by image processing system CalMorph we developed. To systematically compare the variance by 501 traits, we assigned the most appropriate probability distribution models to each trait, and applied the generalized linear model (GLM) to the morphological phenotypes. Comparing the variance of each trait between natural strains and gene deletion mutants, we found that the gene deletion mutants had higher morphological variance than the natural strains in 99% of the morphological traits. Thirty-six gene deletion mutants conferred significant morphological changes beyond that of the natural strains, revealing the importance of the genes with high genetic interaction and specific cellular functions for species conservation. Based on the morphological analysis, we discovered gene deletion mutants whose morphologies were not seen in nature. Our multivariate approach to the morphological diversity provided a new insight into the evolution and species conservation of yeast.

PS16-20: Evolutionary conservation of the N-alpha-terminal acetyltransferase C (NatC) complex from yeast to human**Camilla Osberg¹, Sandra Ninzima², Henriette Aksnes², Thomas Arnesen¹**¹*Department of Molecular Biology, University of Bergen, Department of Surgery, Haukeland University Hospital, Norway; ²Department of Molecular Biology, University of Bergen, Norway*

The yeast Golgi protein Arl3 is dependent on interaction with the transmembrane protein Sys1 for correct localization to the Golgi and this interaction-mediated targeting requires N-terminal acetylation of Arl3 by the NatC complex. This is one of several examples suggesting N-terminal acetylation to have a function in protein targeting to membranes, although this has been difficult to pinpoint as a general mechanism. The NatC complex acts co-translationally and is composed of the catalytic subunit Naa30 and the auxiliary subunits Naa35 and Naa38, although Naa38 is dispensable for correct Arl3 localization. Here, we utilized the known Arl3 mislocalization phenotype of NatC-lacking yeast as a model system and discovered a conserved functional activity between *S. cerevisiae* and human NatC. Human Naa30 was able to restore Arl3 Golgi membrane localization in the absence of yNaa30, but only in the presence of yNaa35. Co-expressed hNaa30 and hNaa35 generated a functional NatC complex in yeast naa35Δ cells, thus indicating Naa35 to serve as a ribosomal anchor of NatC. A recent study described NatF/Naa60 as the first organellar NAT and suggested its possible post-translational activity on the Golgi. Using the yeast Arl3 localization model system, we here investigated this hypothesis and observed that human Naa60, expressed in Arl3-GFP naa30Δ yeast, localized to the Golgi and was able to N-terminally acetylate Arl3 and rescue its Golgi-localization. Thus we here challenge the established dogma of N-terminal acetylation as a mainly co-translational event and address the evolutionary conservation of NatC from yeast to human.

PS16-21: The prokaryotic origin of mitochondria studied by the metalloid tellurium as a valuable tool**Graziano Pizzolante¹, Paola Pontieri², Mario De Stefano³, Roberta Romano², Pietro Alifano², Norio Gunge⁴, Isamu Miyakawa⁵, Nobundo Sando⁵, Luigi Del Giudice²**¹*Department of Biological and Environmental Sciences and Technology, University of Salento, Lecce, Italy;*²*Institute of Biosciences and Bioresources-UOS Portici,CNR, Portici (NA) c/o Dipartimento di Biologia, Sezione di Igiene, Napoli, Italy; ³Department of Environmental Sciences, Second University of Naples, via A. Vivaldi 43, 81100 Caserta, Italy; ⁴Sojo-University, Ikeda 4-22-1, Kumamoto 860-0082, Japan; ⁵Department of Biology, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan*

Mitochondria are eukaryotic organelles which contain the own genetic material and evolved from free-living eubacteria, namely hydrogen-producing Alphaproteobacteria [1]. Since 1965, biologists provided, by research at molecular level, evidence for the prokaryotic origins of mitochondria[2]. The use of new tools to evidence the prokaryotic origin of mitochondria could be useful to gain an insight into the bacterial endosymbiotic event that resulted in the permanent acquisition of bacteria, from the ancestral cell, that through time were transformed into mitochondria. When grown in the presence of potassium tellurite (K_2TeO_3), bacteria form black colonies due to the deposition of intracellular crystals of elemental tellurium (Te^0)[3]. By transmission electron microscopy (TEM) we investigated the capacity of both proteobacterial and yeast cells during their growth in the presence of increasing amount of tellurite to precipitate K_2TeO_3 into Te^0 forming large deposits either along the proteobacterial membrane or along the yeast cell wall and mitochondria. Since the mitochondrial inner membrane composition is similar to that of proteobacterial membrane, in the present work we evidenced the black tellurium deposits on both, cell wall and mitochondria of ρ^+ and respiratory deficient ρ^- mutants of yeast. This finding opens new scenarios on conundrum of tellurite reduction and toxicity. The research was supported by Compagnia di San Paolo special grant "iniziativa" to L. Del Giudice.

[1] Gray et al. (2004) *Annu. Rev. Genet.* 38, 477-524; [2] Nagai, S. (1965) *J. Bacteriol.* 90, 220-222; [3] Taylor (1999) *Trends Microbiol.* 7, 111-115.

Poster Session 16: Yeast comparative and evolutionary biology**PS16-22: Rewiring of transcriptional control of lysine biosynthesis in *Candida albicans*****Yumnam Priyadarshini, Krishnamurthy Natarajan***Laboratory of Eukaryotic Gene Regulation, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India*

How pathogenous and non-pathogenous fungi adapt to their environments is of fundamental importance for effective management of fungal diseases. Indeed rewiring of transcriptional network is an important facet of evolutionary adjustments for survival under various niches. Regulation of lysine biosynthetic pathway in *Saccharomyces cerevisiae* occurs at multiple levels namely the general amino acid control via Gcn4, feedback inhibition of homocitrate synthase activity by lysine, and activation of lysine pathway-specific transcriptional regulator Lys14 by alpha-aminoadipate semialdehyde. Genome annotation revealed that the human fungal pathogen *Candida albicans* genome encodes four ORFs named *LYS14-LYS144*, encoding highly conserved Lys14-like Zn(II)₂Cys₆ DNA-binding domain. The amino acid sequence of the Zn(II)₂Cys₆ domain as well as the complete sequence of each of the four Lys14-like sequences showed similar homology to the *S. cerevisiae* Lys14 amino acid sequence. *GAL1*-driven expression of none of the four *C. albicans* *LYS14*-like sequences complemented the lysine auxotrophy of *S. cerevisiae* *lys14Δ* mutant, and thus a functional Ca*LYS14* ortholog could not be identified. Mutational analyses showed that none of four *C. albicans* *lys14Δ* null mutants showed lysine auxotrophy, and the expression of Lys14-like genes, both at mRNA and protein levels, was also not modulated by lysine in *C. albicans*. In contrast, we demonstrate that Ca*GCN4* is the primary regulator of *LYS* biosynthetic genes as *gcn4Δ* mutant showed lysine auxotrophy, and *LYS2* and *LYS9* expression under amino acid starvation conditions was Gcn4-dependent. Chromatin immunoprecipitation assays showed Gcn4, but not Lys14, occupancy at the *LYS2* and *LYS9* promoters under amino acid starvation conditions. These data provide strong evidence that Gcn4, but not *LYS14*-like genes is the primary regulator of lysine biosynthesis in *C. albicans*. Thus our studies reveal a transcriptional rewiring of the control of lysine biosynthesis in *C. albicans* that is linked to both TF specificity as well as changes in the cis-regulatory elements in the promoters supporting the requirement for co-evolution of cis-elements and TF sequences.

PS16-23: Genetics without crosses: Quantitative trait mapping with CRISPR**Meru Sadhu, Joshua Bloom, Leonid Kruglyak***University of California, Los Angeles, USA*

Linkage mapping is a powerful tool to map genetic variants underlying traits of interest. The principle is to analyze the trait in a cross or family in which the trait segregates, to find chromosomal regions that associate with the trait. This approach relies on recombinations in meiosis to separate the causal variant from the remainder of the genome. Since these recombinations occur randomly, obtaining higher resolution mapping requires ever-larger panels of individuals. This is made even more challenging by variation in recombination activity along the genome; causative variants that reside in recombinational coldspots are especially difficult to interrogate. We have developed a novel method to map trait variation, using programmable mitotic recombinations events triggered by the endonuclease Cas9. Because Cas9 creates DNA double-strand breaks in a highly targetable manner, it can be used to massively enhance linkage analysis resolution in any region of the genome. As a proof of principle, we show that panels of yeast with Cas9-induced mitotic recombinations can be used to map a wide variety of traits. We use this method to map manganese sensitivity to a single causative polymorphism.

PS16-24: Differential gene expression modulates evolutionary trajectories across yeast species**Monica Sanchez, Ivan Liachko, Aaron Miller, Maitreya Dunham***University of Washington, USA*

Both comparative genomics and experimental evolution are powerful tools that can be used to make inferences about evolutionary processes. Together, these approaches provide the opportunity to observe evolutionary adaptation over millions of generations and in response to controlled selective pressures in the laboratory. To test how different species adapt to a constant limited environment, we performed laboratory evolution experiments using multiplexed chemostats to evolve multiple populations of *Saccharomyces cerevisiae* and *Saccharomyces*

uvarum under sulfate limitation for ~200-500 generations and identified differential adaptation through the amplification of different paralogs. The locus containing the high affinity sulfate transporter *SUL1* was amplified in every evolved culture of *S. cerevisiae*. However, in *S. uvarum*, we identified a copy number increase at the *SUL2* locus, which codes for a lower affinity sulfate transporter in *S. cerevisiae*. We determined differential fitness effects of each gene, suggesting that, in *S. uvarum*, *SUL2* may be more important for growth in sulfate limitation. We identified reduced fitness and expression level of chimeric alleles of *SUL1* and *SUL2* from both species that contain the promoter of *SUL1* from *S. uvarum*, suggesting that the divergence in the selective effect of *SUL1* and *SUL2* is due to the changes in the upstream noncoding sequence. In order to better understand divergence of these paralogs across the *sensu stricto* genus, laboratory experimental evolutions in sulfate-limitation were performed in two other species of the *sensu stricto* genus: *S. paradoxus* and *S. mikatae*. Amplification of the *SUL1* locus was detected for both species with no amplification of *SUL2*. Our data show evidence of differential sub-functionalization among the sulfur transporters across the *sensu stricto* genus through recent changes in noncoding sequence. This study highlights differential evolutionary adaptations between multiple diverged yeast species and presents a method for tracking evolutionary path differences in microorganisms.

PS16-25: The sex determination system and the role of chimeric a1/alpha2 heterodimer in the sterility of on allodiploid *Zygosaccharomyces* yeast

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In *Saccharomyces cerevisiae* diploids, the a1/alpha2 protein heterodimer acts as repressor of haploid-specific genes, such as *MATalpha1* and *HO* genes, and allows a/alpha diploid cells to undergo sporulation. The regulatory routes governing the cell type and fate have been not yet studied in the yeasts belonging to the *Zygosaccharomyces rouxii* complex. These yeasts are relevant in foodstuff elaboration and spoilage due to a wide repertoire of tolerances to osmotic stress. Their genetic variability favors phenotypic diversity and adaptation to hostile environments by ectopic recombination of mating-type (MTL) loci through an error-prone switching mechanism. Among them, the allodiploid strain ATCC42981 exhibits hybrid vigor and multi-stress tolerance, but it is unable to undergo sexual reproduction. Aim of this work was to characterize allodiploid strain ATCC42981 for the genetic organization and the transcriptional expression of mating type-like (MTL) loci and HO genes. Genetic dissection of ATCC42981 sex determinants revealed a MATa/MATalpha genotype with a redundant number of partial divergent HMR cassettes. MATa expression locus contained *Z. rouxii*-related *MATA1* and *MATA2* genes, whereas *MATalpha* expression locus contained *Z. sapae*-related *MATalpha1* and *MATalpha2* genes (termed *MATalpha1* and *MATalpha2* copy 2, respectively). Both *MAT* expression loci are linked to phylogenetically congruent *HML* silent cassettes on non-homologous chromosomes. Other than a “hybrid” and redundant three-cassette system, ATCC42981 possesses two divergent HO genes. Consistently with this gene organization, ATCC42981 expressed *MATA1* and *MATalpha2* copy 2 genes under standard conditions, whereas a- and α-idiomorph genes from *HMR* and *HML* cassettes were silent. Differently from *S. cerevisiae* diploids, ATCC42981 did not repress either *MATalpha1* or *HO* gene transcription. Under hypersaline stress (which should induce meiosis in *Zygosaccharomyces* cells by turning on the haploid gene-specific program), ATCC42981 was not able to undergo meiosis and over-expressed *HO* copy 2, *MATA1* and *MATalpha1* copy 2. We hypothesized that the partial incompatibility between ATCC42981 *Z. rouxii*-like a1 and *Z. sapae*-like alpha2 subunits in *MATA1/alpha2* heterodimer causes a defective silencing of haploid-specific genes, including meiosis inhibiting factors, leading to clonality as the only possible reproduction strategy for this allodiploid yeast.

Poster Session 16: Yeast comparative and evolutionary biology**PS16-26: Social wasps are mating nests for yeasts**

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Saccharomyces cerevisiae (Sce) is largely used as a model for a wealth of purposes. The recent availability of genome sequences of a large number of *S. cerevisiae* and *S. paradoxus* (Spa) strains representing the widest known genetic, phenotypic and geographical diversity renewed the interest in the use of these yeasts as models for evolution and ecology studies. Nevertheless, one of the still unanswered questions is whether genetically diverse yeasts mate and recombine in the wild. The yeasts outcrossing was estimated to occur only once every 10⁵ mitotic division, thus confining their reproduction to mitosis and to occasional intra-ascus breeding (inbreeding). Although, the recent observation on larger set of strains of unexpectedly high levels of genetic heterozygosity and prions diffusion called the rarity of outcrossing into question. To outbreed at least two conditions have to occur: *i*) different strains has to simultaneously inhabit the same area, *ii*) they have to face environmental oscillations favouring sporulation (because natural yeasts are usually diploid) followed by germination. Social wasps have been shown to bear yeast cells all year long and feeding on sources that are potentially inhabited by different *Saccharomyces* spp. strains, thus representing a potential incubator for different yeast cells to meet and mate. Here we show that the intestine of social wasps favours the mating of different yeast strains and species by providing a sequentiality of environmental conditions prompting the sporulation and germination of *S. cerevisiae* and making heterospecific mating the only option for *S. paradoxus* to survive. Our results open a new perspective introducing insects as unaware players in the evolution of *Saccharomyces* spp. yeasts. *Saccharomyces* spp. yeasts could prefer sexual reproduction to react to the environment changes occurring within the wasp intestine and in the continuous flux from the wasp to the environment and vice-versa.

PS16-27: The impact of high gene overexpression on genetic stability of *Saccharomyces cerevisiae*

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Gene overexpression can impair different fitness components, one of them is the stability of genome. A standard approach to assay genetic stability is to screen for the loss of heterozygosity (LOH). It is typically done by constructing a diploid yeast strain with a heterozygous locus containing a recessive marker. Appearance of the latter marks loss of its dominating counterpart. We used a collection of diploid strains with the loci *CAN1/can1* and *MET6/met6::kanMX4* (located on the opposing arms of chromosome V). Overexpression of single genes was facilitated by using a multicopy 2μ overexpression plasmid with an inducible *GAL1* promoter. Following overexpression, strains were grown on medium containing canavanine to score the frequency of canavanine resistant colonies (lacking functional *CAN1*). Of about 6000 strains tested, several hundred showed an increased rate of LOH; a decreased rate of LOH was also found, although somewhat less frequently. Repeating the assay allowed to narrow the search to a smaller number of strains and to identify functional categories of genes which control LOH. The rate of LOH was enhanced by overexpression of genes involved in mitotic cell division, especially sister chromatid cohesion; it was decreased by overexpression of genes engaged in DNA repair, especially double-strand break repair. It was previously shown that null mutations can alter the rate of LOH. Our findings show that it is also sensitive to single gene overexpression.

PS16-28: Adaptation to high ethanol reveals complex evolutionary pathways

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One of the main quests in biology is the identification of mutations that define a complex, polygenic trait. How do multiple genes interact to generate a certain phenotype? How do various (combinations of) mutations gradually arise during evolution and adaptation? Are there multiple paths leading to the same phenotype? Experimental evolution is a valuable tool that generates insight into these issues. Tolerance to high levels of ethanol is an ecologically and industrially relevant phenotype of microbes, but the molecular mechanisms underlying this complex trait remain largely unknown. Here, we use experimental evolution of isogenic yeast populations of different initial ploidy to study adaptation to increasing levels of ethanol. Evolved lineages showed a significant increase in ethanol tolerance compared to ancestral strains. High coverage whole-genome sequencing of more than 30 evolved populations and over 100 adapted clones isolated throughout a two-year evolution experiment revealed how a complex interplay of different evolutionary mechanisms led to higher tolerance, including de novo single nucleotide mutations, extensive copy number variation and ploidy changes. Although the specific mutations differ between different evolved lineages, network analysis reveals shared themes at the level of functional modules. Moreover, by combining an allelic replacement approach with high-throughput fitness measurements, we could identify several SNPs that arose in our adapted cells previously not implicated in ethanol tolerance and that significantly increased ethanol tolerance when introduced into a non-tolerant background. Interestingly, one of the genes mutated, VPS70, was recently identified as a causative gene for ethanol tolerance in an industrial bio-ethanol strain. Taken together, our results show how adaptation to a complex stress such as increasing ethanol levels involves an interplay of different evolutionary mechanisms. In addition, our study highlights the potential of experimental evolution to identify mutations that are of industrial importance.

PS16-29: Hidden in plain sight: the eukaryotic conserved unstudied proteins and a framework for their classification and characterisation

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Proteins conserved widely among eukaryotes play fundamentally important roles in the shared, basic mechanisms of life. The roles of many broadly conserved proteins remain unknown, however, despite almost a century of gene- and gene product-specific genetic and biochemical investigation. Even the recent emergence of genome-wide experimental techniques and the availability of near-complete protein inventories for many intensively studied eukaryotic model species have shed light on the functions of few previously uncharacterised conserved proteins. Because the success of many endeavours in basic and translational research, including drug discovery, metabolomics, and systems biology, depends critically on comprehensive representation of conserved functions, a more complete understanding of protein components conserved throughout eukaryotes would have far-reaching benefits for biological research in many species and on a wide range of scales. To identify priority targets for experimental investigation, PomBase provides an inventory of fission yeast proteins that are conserved among eukaryotes but whose broad biological roles remain unknown. A broad functional classification of the known proteome using a selection of Gene Ontology biological process categories ("GO Slim") has revealed correlations with features such as subcellular localization and morphological phenotype. Combining available data from genome-wide phenotype and localization experiments with insights from the functional classification of known proteins facilitates prediction of biological roles, and thereby guides specific experimental characterisation of unknown proteins.