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Keynote Lectures

[OL] Molecular mechanism and physiological role of autophagy

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Every cellular component is maintained by balance between synthesis and degradation. Cellular degradation process is highly regulated and plays critical roles in cell physiology. There exist two major pathways of intracellular degradation, lysosome/vacuole- and ubiquitin/proteasome- systems. The former is mediated mainly via autophagy, facilitates principally bulk non-selective degradation. Since discovery of lysosome and coining autophagy as self-eating process by C. de Duve, for long time not much progress had been made about its molecular mechanism.

More than 28 years ago I first found by light microscopy that the yeast, S. cerevisiae, induces massive protein degradation within the vacuole under nutrient starvation. Electron microscopy revealed that membrane dynamics during the process is topologically the same as known macroautophagy in mammals. Taking advantage of the yeast system, we succeeded in isolation of many autophagy-defective mutants. Now we know that 18 ATG genes are essential for starvation-induced autophagy. These Atg proteins function concertedly in the sequestration of cytoplasmic constituents into a specialized membrane structure, the autophagosome. The Atg proteins consist of six functional units, including an Atg1 kinase complex, the PI3 kinase complex and two unique ubiquitin-like conjugation systems. Soon we found that these core ATG genes are well conserved from yeast to mammals. The identification of ATG genes completely changed the landscape of autophagy researches. By genetic manipulation of ATG genes in various organisms and specific organ or individuals, revealed so broad range of physiological functions of autophagy. Not only nutrient recycling, autophagy plays critical roles for intracellular clearance by elimination of harmful proteins, damaged organelles, and bacteria escaped in the cytoplasm. It is getting clear that autophagy is relevant to many diseases such as neurodegeneration, infection and cancer. Now autophagy has become so popular field in cell biology.

We are attempting to elucidate the mechanisms of the unique membrane dynamics during autophagy by studying the structure and function of Atg proteins. Even in yeast autophagy there are many fundamental questions remain to be answered. Further comprehensive and biochemical analyses are required from various points of view. Present our knowledge on the molecular mechanism and physiological roles of autophagy in yeast will be presented and discussed.

Keywords: *ATG, autophagy, vacuole, protein degradation*

[KL1] A network of molecular interactions restricts stable preinitiation complex assembly to optimal translation start codons in vivo

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In eukaryotic translation initiation, a preinitiation complex (PIC) comprised of the 40S ribosomal subunit, initiation factors 1, 1A and 3, and a ternary complex (TC) of eIF2-GTP and initiator tRNAi attaches to mRNA and scans the leader for an AUG codon in optimal context. eIF1 stimulates recruitment of TC with tRNAi bound in a state (POUT) suitable for scanning, and opposes transition to a more stable PIN state at the start codon, necessitating eIF1 release on AUG recognition. Structures of reconstituted PICs reveal an open 40S conformation at a near-cognate AUC codon, and a more closed configuration at AUG in which eIF1 shifts on the 40S and its β-hairpin loops are distorted to avoid clashing with tRNAi. Substitutions in eIF1 loop-2 that remove ionic repulsion or create attraction with tRNAi increase initiation at UUG codons (Sui- phenotype) and AUGs in poor context in vivo, and stabilize TC binding at UUG start codons in reconstituted PICs. Thus, the loop-2/tRNAi clash destabilizes the PIN state to disfavor suboptimal start codons. eIF2β contacts eIF1 and tRNAi exclusively in the open complex, and substitutions at the eIF2β/eIF1 interface confer Sui- phenotypes, indicating eIF1:eIF2β interactions also impede rearrangement to PIN at suboptimal start sites. In contrast, the unstructured N-terminal tail (NTT) of eIF1A contacts the codon:anticodon helix only in the closed/PIN state, and substituting its basic residues suppresses initiation at UUG codons (Ssu- phenotype) and destabilizes TC binding at UUG codons in vitro. Ribosome profiling of the eIF1A-R13P mutant reveals decreased initiation at AUGs in poor context genome-wide, implicating the NTT in selecting suboptimal start codons. Similar genetic/biochemical findings identify roles for ribosomal proteins at the mRNA exit (uS7) and entry (uS3) channels in stabilizing the closed/PIN conformation via contacts with eIF2α (uS7) or mRNA (uS3), to enable recognition of suboptimal start sites. eIF3 is a central player in PIC recruitment to mRNA, and we used model mRNAs lacking contacts with the 40S entry or exit channels to identify a critical role for eIF3a in stabilizing PIC:mRNA interactions at the exit channel, and an ancillary role at the entry channel that is functionally redundant with uS3 residues that contact mRNA. Thus, multiple interactions within the PIC serve to stabilize the open or closed states and set the proper stringency level for initiation at non-optimum start codons in vivo.

Keywords: *translation, initiation, eIF1, eIF2, eIF3, ribosome*

[KL2] Anti-Prion Systems in S. cerevisiae

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[URE3] and [PSI+] are amyloid-based prions of Ure2p and Sup35p, respectively. The infectious amyloid filaments of Ure2p and Sup35p each have an in-register parallel β-sheet architecture, with the β-sheets folded along the long axis of the filaments. This architecture naturally suggests a conformational templating mechanism, explaining how these proteins can act as genes (Wickner RB, et al. Microbiol Mol Biol Rev 79: 1-17 (2015)).

Overproduction of Btn2p or Cur1p cures any [URE3] prion variant, and at normal levels cures most prions arising in their absence. They thus constitute an anti-prion system protecting the cells from the known detrimental effects of all [URE3] variants. Btn2p curing occurs by collecting the Ure2p amyloid aggregates at one place in the cell, resulting in frequent prion - free daughter cells (Kryndushkin et al. EMBO J 27: 2725 (2008); Wickner et al. PNAS 2014 pnas.1409582111). Normal-level Btn2p curing works on variants with lower seed number than those whose curing requires overproduction of Btn2p. The disaggregating chaperone Hsp104 is needed by most yeast amyloid-based prions to generate new seeds, but overproduction of Hsp104 cures any [PSI+] variant. Mutations in the N-terminus of Hsp104, such as T160M, eliminate the overproduction - curing function of Hsp104, without affecting its prion promoting activity. We found that most [PSI+] variants arising in an hsp104T160M strain are eliminated by restoration of just normal levels of the normal protein (Gorkovskiy and Wickner, PNAS, 2017 pnas.1704016114). Thus, like Btn2p and Cur1p action on [URE3], Hsp104 is an anti-prion system working on the generally detrimental and sometimes lethal [PSI+] prion.

We have carried out a general screen for anti-prion systems working on [PSI+] demanding that they work without overexpression, using an approach similar to that developed for Btn2, Cur1 and Hsp104.

Keywords: *prion, amyloid, templating protein conformation, Btn2, Cur1, Hsp104, anti-prion, in-register parallel, beta sheet*

[CL] From Rags to Riches and Back Again – Nitrogen-Responsive Regulation in S. cerevisiae

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It’s critical for yeast cells to manage internal nitrogen homeostasis in the face of continuous, drastic external transitions. Nitrogen Catabolite Repression (NCR) is the overall regulatory mechanism permitting cells to take full advantage of luxurious nitrogenous environments, yet retain the ability to cope with austere ones; all the while maintaining tight intra-cellular homeostasis. Two types of transcription factors participate: (i) Pathway-specific activators whose binary functioning depends on the concentration of a single inducer, e.g. allophanate for allantoin (DAL) transcription. (ii) Pathway-independent, NCR-sensitive GATA-binding activators, Gln3 and Gat1, which respond to overall intra-cellular nitrogen availability. In nitrogen replete conditions the GATA factors are sequestered in the cytoplasm and NCR-sensitive transcription is minimal. As conditions deteriorate, Gln3 relocates to the nucleus dramatically increasing GATA factor-mediated transcription. NCR-sensitive regulation was

originally attributed to TorC1-mediated control of Gln3. However, Gln3 responds to 5 distinct physiological conditions each exhibiting a unique set of regulatory requirements. This, other data and the unique structure of Gln3 allowed us to demonstrate that nitrogen-responsive TorC1 activity only partially accounts for NCR-sensitive regulation. Multiple, previously elusive regulators have now been identified. (i) Uncharged tRNA-activated, Gcn2 kinase-mediated General Amino Acid Control (GAAC). Gcn2 and Gcn4 are required for NCR-sensitive, TorC1-independent nuclear Gln3 localization. Epistasis experiments indicate Gcn2 likely functions upstream of Ure2. Bmh1/2, also required for nuclear Gln3 localization, likely function downstream. Interestingly, overall Gln3 phosphorylation levels decrease upon loss of Gcn2, Gcn4 or Bmh1/2. Further, nuclear import is more complex than previously appreciated and likely occurs in multiple steps. (ii) TorC1 and Gcn2, acting in opposition, are augmented by a third level of intra-nuclear regulation. In high glutamine, Gln3 exits from the nucleus in the absence of binding to its GATA targets in NCR-sensitive promoters. In contrast, as glutamine levels decrease, GATA binding becomes requisite for Gln3 to exit from the nucleus. The concerted actions of these multiple regulatory components impressively illustrate how NCR effectively manages intra-cellular homeostasis in the face of extra-cellular nitrogen environment transitions. GM35642-27.

Keywords: *Nitrogen catabolite repression, Gln3, Ure2, Gcn2, Gcn4, TorC1, rapamycin, nuclear import, nitrogen environments*

Symposia

[S1] Regulation of gene expression

[S1-1] The nuclear pore complex facilitates interchromosomal clustering and epigenetic transcriptional regulation

Brickner Jason

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Yeast has been an excellent model for the conserved interaction between the nuclear pore complex (NPC) and hundreds of chromosomal loci. This interaction both affects transcriptional regulation and can lead to epigenetic transcriptional memory, poising inducible genes for faster reactivation for several generations after repression. I will discuss our work defining the molecular mechanisms that control gene positioning, interchromosomal clustering and transcriptional memory in association with the NPC. We find that localization at the nuclear periphery and interaction with the NPC is controlled by transcription factors and that this often leads to interallelic and intergenic clustering. A majority of transcription factors are capable of mediating targeting to the NPC by two major pathways, one that operates when genes are active and another that operates when they are poised. Poising results from the repurposing of factors associated with transcription (Mediator and COMPASS) to promote the poised state. Permissive chromatin changes lead to binding of RNA polymerase II preinitiation complex, bypassing the rate-limiting step in transcription.

Keywords: *nuclear architecture, epigenetics, transcription, chromosomes, transcription factor*

[S1-2] New Insights Into Translational Control By eIF2B

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The GTP-binding protein eIF2 acts to deliver initiator tRNA (Met-tRNAi) to the ribosome to begin protein synthesis on all cytoplasmic mRNAs. In common with other G proteins eIF2 is active when bound to GTP and not when bound to GDP. This switch enables tight control of protein synthesis. For example in times of stress global protein synthesis is repressed because Gcn2 phosphorylates eIF2α and this inhibits eIF2 activation repressing translation initiation. Stress-responsive RNAs, such as GCN4 can escape this repression. Key to controlling the activity of eIF2 are translation factors eIF2B and eIF5, thought to primarily function with eIF2-GDP and eIF2-GTP/Met-tRNAi complexes, respectively, as GEF and GAP

proteins. Here I will outline work done by my lab that has identified new roles for eIF5 and eIF2B that have enabled us to develop new models for the interplay and competition between these factors for interaction with eIF2. Together these lead to either eIF2 activation or its tight control by eIF2 phosphorylation in response to stress.

Keywords: *eIF2, translational control*

[S1-3] Translation fidelity: how stop codons can be read by tRNA?

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The genetic code can be read by cognate but also near cognate tRNAs. This flexibility is assumed to be conferred mainly by a mismatch between the third base of the codon and the first of the anticodon (so called wobble position). However this simplistic explanation often underestimates the importance of nucleotide modifications in the decoding process. Taking advantage of a system where only near cognate tRNA can decode a specific codon, we addressed the role of six modifications found in the anticodon, or adjacent nucleotides, of tRNA Tyr, Gln, Lys, Trp, Cys and Arg in Saccharomyces cerevisiae. We show that almost systematically, the modification confers to these tRNAs the ability to act as a near cognate tRNAs on stop codons, without noticeable impact on their ability to decode cognate or near-cognate sense codons. These findings reveal an important role of modifications for tRNA decoding.

Keywords: *Translation, Stop codon, tRNA*

[S1-4] Translational control in the cell cycle

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Translational control during cell division determines when cells start a new cell cycle, how fast they complete it, the number of successive divisions, and how cells coordinate proliferation with available nutrients. Translational control in human cells arrested in the cell cycle has been examined previously by ribosome profiling. However, these earlier studies did not query the translational efficiencies of mRNAs in cells progressing synchronously through the mitotic cell cycle, while preserving the coupling of cell division with cell growth. We recently reported comprehensive ribosome profiling of a yeast cell-size series from the time of cell birth, to identify mRNAs under periodic translational control [EMBO J. 2017 36(4):487-502]. We found coordinate translational activation of mRNAs encoding lipogenic enzymes late in the cell cycle including Acc1p, the rate-limiting enzyme acetyl-CoA carboxylase. An upstream open reading frame (uORF) confers the translational control of ACC1 and adjusts Acc1p protein levels in different nutrients. The ACC1 uORF is relevant for cell division because its ablation delays cell cycle progression, reduces cell size, and suppresses the replicative longevity of cells lacking the Sch9p protein

kinase regulator of ribosome biogenesis. These findings establish a novel and unexpected relationship between lipogenesis and protein synthesis in mitotic cell divisions. Furthermore, in unpublished work, we found that two conserved proteins that govern duplication of the spindle pole body in the G1 phase, Dsk2p and Mps1p, are under strong, uORF mediated, translational control. These findings link molecularly cell growth and protein synthesis with the machinery for chromosome segregation. Finally, we will present preliminary data from ribosome profiling of ribosomal protein (RP) deletions in the cell cycle, identifying mRNAs under translational control that mediate RP paralog-specific effects on cell division and replicative longevity.

Keywords: *cell cycle, translation, ribosome profiling*

[S2] RNA processing and regulation

[S2-1] Investigating role of phosphorylation of the RNA polymerase II in transcription and 3'end formation of functional mRNA

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Reversible phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II) is important for coordinated regulation of transcription and maturation of functional mRNA, yet the molecular mechanism underpinning CTD function remains unclear.

CTD consists of multiple repeats of the consensus sequence (Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7) where it can be phosphorylated on Y1, S2, T4, S5, and S7P. While regulation and function of S2P have been extensively studied, role of T4P as well as kinases and phosphatases responsible for regulation of this recently identified modification are not known. We observe that S2P, T4P and Y1P co-occur on CTD at the end of the transcription cycle in fission yeast suggesting that these marks might be important at this stage. We show that conserved PP1 phosphatase is involved in T4P regulation and lack of its activity leads to genome –wide transcription termination defect. Our findings reinforce the importance of CTD phosphorylation in regulation of Pol II transcription and provide mechanistic insights into CTD function.

Keywords: *RNA polymerase II, Reversible phosphorylation, Pol II transcription*

[S2-2] Formation of Nuclear Messenger Ribonucleoprotein Particles (mRNPs)

Sträßer Katja

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Gene Expression is one of the fundamental processes of life. In eukaryotes, the mRNA is synthesized by RNA polymerase II transcribing the protein-coding genes. Already largely cotranscriptionally, the mRNA is processed, i.e. capped, spliced and polyadenylated, and packaged by binding of RNA-binding proteins (RBPs) into a messenger ribonucleprotein particle (mRNP). Binding of these nuclear RBPs to the mRNA determines essential steps during the later life of the mRNA such as its nuclear export, translation rate and stability. Thus, packaging of the mRNA into an mRNP is an essential step of gene expression.

We use the yeast S. cerevisiae to elucidate the mechanism and function of mRNP packaging because in this model organism all the nuclear mRNP components are – most likely – known. However, the coordinated assembly as well as the changing composition and structure of an mRNP has remained largely enigmatic despite a lot of research in this field. I

will review our current knowledge of mRNP packaging and also report novel insights into this process from recent research of my laboratory.

Keywords: *gene expression, mRNPs, RNA-binding proteins, Saccharomyces cerevisiae*

[S2-3] Fitness Landscape of Yeast U3 snoRNA

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The concept of fitness landscape is a commonly used metaphor in evolutionary biology: evolving populations are represented as sets of points that navigate a genotype space in search of peaks of high fitness. Although the concept has been used for decades, it has long been unclear what an actual fitness landscape looks like. Recent advances in synthetic biology and next-generation sequencing allow experimental investigation of fitness landscapes, and open the way to answering long-standing, fundamental questions in molecular evolution: Which mutations influence function? How do mutations influence function? How do effects of mutations depend on environmental conditions and genetic background?

We are studying these questions using yeast U3 snoRNA as a model system. U3 is an abundant, evolutionarily conserved noncoding RNA, which plays an essential role in ribosome biogenesis. By measuring the effects of 60,000 mutated variants of U3 on yeast growth, we found that the effects of individual mutations were correlated with evolutionary conservation and structural stability. Many mutations had no measurable effect in an otherwise wild-type background, but were deleterious in combination with additional mutations in U3. We also found pairs of compensatory mutations, and used these to predict the secondary structure of the RNA. I will discuss our findings in the context of the recent cryo-electron microscopy structural studies of yeast U3 ribonucleoprotein.

Keywords: *synthetic biology, saturation mutagenesis, molecular evolution, short nucleolar RNA*

[S2-4] P-bodies regulate transcriptional rewiring during DNA 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 degradation and/or storage of mRNAs. We recently discovered that P-bodies form in yeast in response to DNA replication stress induced by HU (hydroxyurea). 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. Here, we aimed to identify mRNAs that are processed by P-bodies during replication stress. First, we performed a transcriptome study on lsm1Δ cells

upon acute HU exposure to identify mRNAs that are stabilized in the absence of a functional P-body-dependent mRNA degradation pathway. Second, we used an SGA-based suppressor screen to identify genes whose expression is toxic in the absence of Lsm1 and Pat1 during replication stress. We found that the transcriptome in lsm1Δ is altered both during normal growth and during replication stress, with more than 800 mRNAs being stabilized in lsm1Δ compared to wild type. Interestingly, we found that inactivation of the coding sequence of 6 of those 800 mRNAs was able to suppress HU sensitivity of lsm1Δ and pat1Δ strains suggesting that these genes encode mRNAs that need to be degraded in a P-body dependent manner upon HU exposure. Among these, we identified YOX1, a gene encoding a transcription repressor critical for the regulation of cell cycle and DNA replication genes. Consistent with P-bodies regulating YOX1 mRNA abundance, we found that YOX1 mRNA localizes to P-bodies and accumulates at P-bodies in the absence of the mRNA exonuclease Xrn1. To gain insight into the role of Yox1 during replication stress, we identified 156 genes that are down-regulated upon YOX1 overexpression. Among this set of targets, we found that de-repression of ALD6, encoding a cytoplasmic acetaldehyde dehydrogenase, is critical for replication stress resistance. Indeed, accumulation of acetaldehyde, the substrate of Ald6, is strongly toxic for cells experiencing DNA replication stress. Together, our data suggest a model where YOX1 mRNA abundance is post-transcriptionally regulated by P-bodies in order to reduce the level of the Yox1 transcription repressor and therefore prevent repression of genes necessary for survival of DNA replication stress.

Keywords: *P-bodies, RNA decay, DNA replication stress, Acetaldehyde*

[S3] Metabolism and stress response

[S3-1] Fructose-1,6-bisphosphate Activation of Ras and Nutrient Transceptor - eIF2B/eIF2 Interaction as Novel Mechanisms in Yeast Nutrient Signaling

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The Ras-cAMP-PKA pathway in yeast mediates nutrient regulation of physiology, growth and development. Glucose addition to deprived cells causes a rapid spike in cAMP, which triggers activation of PKA. The cAMP spike is induced by combined action of the glucose-sensing GPCR, Gpr1, and activation of Ras by glycolytic breakdown of glucose, the mechanism of which has remained unclear. Re-addition of another essential nutrient, like nitrogen, phosphate, sulfate or a metal ion, to growth-arrested glucose-repressed cells deprived for such nutrient, triggers a similar rapid activation of the PKA pathway concomitant with upstart of growth. This activation is not mediated by an increase in the cAMP level, and although ample evidence has been obtained that starvation-induced high-affinity transporters function as transporter-receptors or transceptors in this nutrient activation process, the underlying molecular mechanism has remained unclear. We show that glycolytic activation of Ras is mediated by Fru1,6bisP, acting as metabolic messenger for coupling glycolytic flux to PKA. It is likely mediated by a conserved domain in yeast Cdc25 and Sos, its mammalian homolog, since biolayer interferometry measurements show that Fru1,6bisP stimulates dissociation of the pure Sos1/H-Ras complex with a KD of ±9 mM. This is in the physiological range of the cellular Fru1,6bisP level. Thermal shift assays confirm direct binding of Fru1,6bisP to pure Sos1. Glucose addition to mammalian cells also triggers rapid activation of Ras and its downstream targets MEK and ERK. Investigation of a possible role of protein synthesis initiation in PKA activation during nutrient-induced upstart of growth by addition of nitrogen, phosphate, sulfate or a metal ion to appropriately-starved cells, revealed that nutrient transceptors physically bind in vitro to eIF2B and eIF2 subunits. Bimolecular fluorescence complementation experiments with half-citrin tagged constructs confirm the interaction in vivo, which apparently occurs at multiple membranes. This suggests the concept of ‘startosome’, a membrane system controlling the start of protein synthesis. Differential nutrient transceptor-eIF2B/eIF2 interaction allows to design the first molecular model explaining how cells can detect with a common mechanism the absence of any single essential nutrient among the presence of all other essential nutrients, so as to downregulate protein synthesis and arrest cell cycle progression.

Keywords: *Nutrient signaling, Ras/cAMP/PKA pathway, Glycolytic activation of Ras, Yeast/mammalian conservation, Nutrient transceptors, Initiation of protein synthesis, eIF2B/eIF2*

[S3-2] Metabolic differentiation: Role in ageing and long-term survival of yeast colonies

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As with multicellular organisms, yeast and other single-celled microbes are able to differentiate when growing within organized multicellular communities such as colonies and biofilms. According to their 3-D architecture and presence of specific features, two major types of Saccharomyces cerevisiae colonies can be distinguished: structured biofilm colonies, formed by wild strains and smooth colonies, formed by laboratory strains that are adapted to plentiful conditions(J Cell Biol, 194: 679, 2011; Mol Cell 46:436, 2012; Cell Cycle 14: 3488, 2015). Differentiated cells, forming yeast colonies initiate various processes of metabolic reprogramming, gain specific properties, fulfill specific tasks and are able to mutually interact. Signaling molecules, low Mw metabolites and waste products released by cell subpopulations that are specifically localized within the structure further contribute to colony diversification and to differences in longevity of cells undergoing chronological ageing. Unusual combinations of activities of nutrient-sensing signaling pathways and other processes participating in cell reprogramming are involved in development of features, characteristic of different types of stationary-phase colony cells. Recently, we provided evidence that mitochondria play important roles in these processes (Oncotarget 7: 15299, 2016). Mitochondria of differentiated cell-types within smooth colonies differ significantly in numerous parameters and activate three distinct pathways of retrograde signaling that contribute to specific metabolic reprogramming of particular cells via regulation of expression of different metabolic proteins and transporters. These pathways differ in both upstream and downstream participating proteins. Mitochondrial functions seem to be different in smooth and biofilm colonies. This work was supported by GACR 15-08225S and 13-08605S and COST LD15129.

Keywords: *yeast colonies and biofilms, cell differentiation, mitochondrial signaling*

[S3-3] From its origins to the modern metabolic network

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Genome-metabolism interactions enable cell growth, but so far only a relatively small fraction of the genome has been annotated to operate in metabolism. To probe the extent of these interactions and delineate their functional contributions, we quantified the Saccharomyces amino acid metabolome in all strains of the genome-spanning knock-out

collection that can be rendered prototrophic and be grown on minimal media. Over one-third of coding genes, in particular those important for chromatin dynamics, translation, and transport, contribute to biosynthetic metabolism. Specific amino acid signatures characterize genes of similar function. This enabled us to exploit functional metabolomics to connect metabolic regulators to their effectors, as exemplified by TORC1, whose inhibition in exponentially growing cells is shown to match an interruption in endomembrane transport. Providing orthogonal information compared to physical and genetic interaction networks, metabolomic signatures cluster more than half of the so far uncharacterized yeast genes and provide functional annotation for them. A major part of coding genes is therefore participating in gene-metabolism interactions that expose the metabolism regulatory network and enable access to an underexplored space in gene function.

Keywords: *amino acid, metabolom, Saccharomyces, gene-metabolism interactions, TORC1*

[S3-4] Evolutionary Adaptation to a Foreign Hsp90 in the Budding Yeast Saccharomyces cerevisiae

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Heat shock protein 90 (Hsp90) is a molecular chaperone essential for cell viability in all eukaryotic cells. In yeast cells, the whole proteome is influenced profoundly when the Hsp90 activity is reduced. Despite the pivotal role that Hsp90 plays in life, our orthologue replacement assays revealed incompatibility between the Yarrowia lipolytica (Yl) HSP90 and the Saccharomyces cerevisiae genome, suggesting that its interaction network has changed through evolution. To understand how Hsp90 diverged in different species and the underlying driving force, we evolved the Yl-HSP90-carrying S. cerevisiae strain in multiple independent lineages in the normal growth condition (YPD 28°C). After 2,200 generations of evolution, we found that other than improving fitness in the normal condition, different evolved clones gained additional growth advantages in various stress conditions, indicating that they have adapted through different evolutionary trajectories. We sequenced the evolved clones and found that many mutations occurred in Hsp90 clients or client-related proteins. Moreover, several genes involved in protein homeostasis were mutated, suggesting that cells could relieve the Hsp90 defects by enhancing alternative pathways. Bulked segregate analysis was performed in three evolved clones to identify the mutations that have major contributions. In total we identified 10 mutations and confirmed the effect by reconstituting them in ancestral strains. Our results provide the potential explanation for the divergent evolution of Hsp90-interaction network and demonstrate the possible compensatory mutational paths that cells could apply when an essential network is perturbed.

Keywords: *Hsp90, experimental evolution, adaptive mutation*

[S4] Organelle dynamics

[S4-1] Lipid droplets and the ER membrane – a mysterious connection

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Lipid droplets serve to store metabolic energy in form of neutral lipids, particularly triacylglycerol and steryl ester. These neutral lipids are formed by integral membrane enzymes of the endoplasmatic reticulum and are then deposited in lipid droplets from where they can be released again by the action of lipases. While there is growing evidence that early steps of lipid droplet formation occur at or within the ER membrane, later stages of droplet biogenesis are less well understood. We discuss different models of droplet formation and provide experimental evidence that lipid droplets stay closely associated with the ER membrane, possibly throughout their life cycle. Such a close, but topologically still ill-defined association between the ER membrane and lipid droplets is likely to allow for a continuous exchange of membrane anchored proteins as well as lipids between the two compartments.

Keywords: *Lipid droplets, Endoplasmatic reticulum, membrane, neutral lipids, cell compartments*

[S4-2] ER-PM Contacts: Principals of PI Kinase Signaling and Membrane Organization

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The plasma membrane (PM) is highly dynamic and undergoes extensive remodelling during regulated exocytosis and endocytosis. The PM is also spatially organised into distinct domains, including cortical endoplasmic reticulum (ER)-associated PM zones and ER-free PM zones. ER-PM contacts are recognised as important sites for non-vesicular calcium and membrane lipid transport, while exocytic and endocytic vesicular trafficking events are thought to take place exclusively in ER-free PM zones. These two PM domains have been considered to be functionally and spatially uncoupled. Our work is revealing vital roles for inter-organelle cross talk between the ER and PM in membrane trafficking pathways. We are addressing how the architecture of ER-PM contacts is regulated and how distinct ER-PM contacts govern PM domain organization and dynamics. We will present our recent findings on roles for membrane lipid dynamics taking place at ER-PM contacts in the control of phosphoinositide (PI) kinase regulatory networks during regulated exo- and endocytosis.

Keywords: *membrane organization, organelle dynamics, PI kinase signaling*

[S4-3] Never Walk Alone – Contact Sites between Lipid Droplets and Other Organelles.

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Lipid droplets store lipids and thus serve both as an energy reservoir and as a source of building blocks for the organellar membrane systems. Therefore, lipid droplet biology depends on tight communication with other organelles. One important way of interorganellar communication is by formation of contact sites, places where the surfaces of different organelles are actively positioned in very close proximity to each other. We can detect distinct subpopulations of lipid droplets at the surface of virtually all other organelles in S. cerevisiae that appear to be tethered to their surrounding membranes.

We find that a lipid droplet subpopulation positioned adjacent to the NVJ, the contact site between the nucleus and the vacuole/lysosome, is equipped with a unique surface proteome. In an attempt to identify molecular factors determining the identity of these lipid droplets, we screened ~6000 mutants for mislocalization of a subpopulation marker, Pdr16, and identified Ldo45 (lipid droplet organization protein of 45 kDa) as a crucial targeting determinant. Ldo45 is derived from a splicing event connecting two adjacent genes (YMR147W and YMR148W/OSW5/LDO16). Ldo proteins are evolutionary conserved proteins that cooperate with the contact site component seipin, and determine lipid droplet identity by defining their positioning and surface protein composition. Our results suggest a contact site based mechanism to imprint functional specialization of organelles, opening a door to deeper understanding of metabolic decisions in the cell.

Keywords: *Lipid droplet, Organelle contact site, Cellular organization*

[S4-4] Functional Mapping of Yeast Genomes by Saturated Transposition

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Saccharomyces cerevisiae is a powerful model for systems genetics. We present a versatile, time- and labor-efficient method to functionally explore the yeast genome using saturated transposon mutagenesis coupled to high-throughput sequencing. SAturated Transposon Analysis in Yeast (SATAY) allows one-step mapping of all genetic loci in which transposons can insert without disrupting essential functions. SATAY is particularly suited to discover loci important for growth under various conditions.

SATAY (1) reveals positive and negative genetic interactions in single and multiple mutant strains, (2) can identify drug targets, (3) detects not only essential genes, but also essential protein domains, and (4) generates null and other informative alleles.

This method allowed us to identify genes acting in a pathway that uses vacuolar components to bypass ER-mitochondria contact sites. Moreover, in a screen for rapamycin-resistant mutants, we identified Pib2 as a regulator of TORC1. We show that Pib2 possesses two antagonistic TORC1-activating and -inhibiting activities located on opposite ends of the protein. Thus, SATAY allows to easily explore the yeast genome at unprecedented resolution and throughput.

Keywords: *Transposon, Screens, Next-Generation Sequencing, ER, Mitochondria, Vacuole, ERMES, PIB2, TORC1*

[S5] Cellular strategies of protein quality control

[S5-1] Yeast as a Model to Understand Fundamental Mechanisms of Protein Quality Control

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The past two decades have witnessed a paradigm shift in our understanding of cellular protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, we now know that in the crowded environment of cells newly-synthesized polypeptides depend on molecular chaperone proteins to reach their folded states efficiently and at a biologically relevant time scale. Assistance of protein folding is provided by different types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. Once folded, many proteins continue to require chaperone surveillance to retain their functional states, especially under conditions of cell stress. Failure of the chaperone machinery to maintain proteostasis, i.e. the conformational integrity and balance of the cellular proteome, facilitates the manifestation of diseases in which proteins misfold and form toxic aggregates. These disorders include Parkinson's, Huntington's and Alzheimer's disease.

We are using yeast as a model to study the mechanism and consequences of protein aggregation. A focus of ongoing research is on understanding ribosome quality control (RQC). Defects in RQC result in toxic aggregation of nascent polypeptides that stall during translation on ribosomes. These aggregates have features resembling those associated with neurodegenerative disease.

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Science. 2016 Jul 1;353(6294):aac4354. doi: 10.1126/science.aac4354. Review. PMID: 27365453

Choe YJ, Park SH, Hassemer T, Körner R, Vincenz-Donnelly L, Hayer-Hartl M, Hartl FU. Failure of RQC machinery causes protein aggregation and proteotoxic stress. Nature. 2016 Mar 10;531(7593):191-5. doi: 10.1038/nature16973. Epub 2016 Feb 29. PMID: 26934223

Park SH, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, Hayer-Hartl M, Hartl FU. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone.

Cell. 2013 Jul 3;154(1):134-45. doi: 10.1016/j.cell.2013.06.003. Epub 2013 Jun 20. PMID: 23791384

Keywords: *protein folding, protein aggregation, ribosome quality control, molecular chaperones*

[S5-2] Proteostasis function and disfunction: the delicate art of maintaining a healthy proteome

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Correct protein folding and quality control are essential for normal cellular function. The accumulation of misfolded proteins is emerging as central to a wide range of disease states, including many neurodegenerative disorders such as Huntington's and Prion Disease. A complex network of molecular chaperones facilitate protein folding and assembly and monitor all aspects of protein homeostasis. Chaperones assist the folding of newly translated and stress-denatured proteins, as well as affects protein quality control.

Our research investigates the mechanisms and pathways by which chaperones carry out these diverse functions. Systems approaches identified a chaperone network linked to the protein synthesis apparatus assists protein biogenesis. The emergence of this translation-linked chaperone network likely underlies the elaborate co-translational folding process necessary for the evolution of larger multidomain proteins characteristic of eukaryotic cells. A stress-inducible chaperone network protects cells from environmental stress and assists quality control. These chaperones also communicate with the ubiquitin-proteasome pathway to clear misfolded proteins from the cell. Protein quality control in the eukaryotic cytosol relies on the sequestration of misfolded cytosolic proteins in specific quality control compartments. Our studies of chaperone function provide a framework to understand the link between protein misfolding and human disease.

Keywords: *Chaperone, Protein Quality Control, Aggregation, misfolding*

[S5-3] Protein Aggregation During Ageing: Damage or Adaptation?

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Since several decades, budding yeast has proven to be a useful system to study the process of replicative ageing in eukaryotes. Indeed, yeast cells divide constitutively asymmetrically by growing a future daughter cell in the form of a bud at the surface of the mother cell. Interestingly, whereas the mother cell is ageing over time as established by the fact that it can only bud a limited number of times, the large majority of the daughters are born rejuvenated and enjoy the same lifespan potential as their mothers, irrespective of the age of their mother when they were produced. Interestingly, several hallmarks of ageing are conserved from yeast to metazoans, including the accumulation of protein aggregates in the ageing mother cell. Here I will report on our efforts to understand what these aggregates are, how they might contribute to ageing and how they are confined into the mother cell.

A widely shared view about age-associated protein aggregates is that they are formed due to protein misfolding and a decay in protein quality control as the cell age. Investigating this possibility, we have acquired hints that it might not always be the case. Indeed, cells carrying such an aggregate show no deficit in resorbing the Q-bodies forming upon heat-

shock comparted to young cells. Similarly, they show no defect in ubiquitin-mediated protein degradation, excluding the possibility that aggregate form due to a functional weakening of this degradation pathway. In contrast, we have accumulated evidence that yeast cells aggregate specific proteins into super-assemblies in response to external signals as a mean to store information and adapt to environment. These protein aggregation processes contribute to ageing of the cell. I will discuss how they might do so, and how the mother cell ensures that these aggregates are not passed on to the next generation.

Keywords: *Protein aggregation, ageing, asymmetric segregation, mnemons, adaptation, cellular memory*

[S5-4] Genetic dissection of an evolutionary conserved pathway regulating protein degradation

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Timely elimination of misfolded and damaged proteins is essential to maintaining cellular protein homeostasis. A functional decline in the proteolytic capacity of a cell leads to the accumulation of highly toxic protein aggregates that underlies the development of protein misfolding diseases such as Alzheimer's and Huntington's disease. The ubiquitin proteasome system (UPS) is the main cellular pathway for protein degradation where proteins modified by specific ubiquitin (Ub) signals such as K48-linked polyUb are targeted for degradation by the proteasome. We recently discovered a new DUB family, named MINDY, in mammalian cells. Interestingly, two members of this family are conserved down to yeast, and all MINDY DUBs are highly selective at cleaving K48-linked polyUb.

To understand the cellular role of this uncharacterized family of DUBs, we used S. cerevisiae, a powerful tool in the study of pathways regulating protein quality control and homeostasis. Being selective at cleaving K48-linked polyubiquitin, we hypothesized that MINDY DUBs are negative regulators of protein degradation. Indeed, yeast strains lacking MINDY (miyΔ) exhibit increased proteolytic capacity as assessed by degradation of model substrates. Excitingly, our results reveal that miyΔ strains not only have an extended chronological LifeSpan (CLS) but also exhibit an improved replicative LifeSpan. A decline in the proteolytic capacity of a cell is known to result in the accumulation of toxic protein aggregates, one of the underlying causes of ageing and age-related proteinopathies. Indeed, while wild type yeast cells accumulate protein aggregates with age, aged miyΔ cells have hardly any detectable protein aggregates.

In summary, our exciting results reveal that MINDY DUBs are hitherto unstudied important regulators of proteostasis. Further, by studying MINDY DUBs in human cells, my work reveals that the mechanism of action is evolutionarily conserved. Importantly, our results reveal that MINDY DUBs maybe an attractive therapeutic target in neurodegeneration.

Keywords: *Ubiquitin proteasome system, Deubiquitinase, protein misfolding, Proteinopathy*

[S6] Cell cycle and cell fate

[S6-1] The Saccharomyces cerevisiae NADPH oxidase, YNO1, and its Role in Regulating the Actin Cytoskeleton.

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YNO1 (YGL160w) is the only known S. cerevisiae NADPH oxidase. As we have shown in a previous publication (1), overexpression of this enzyme leads to superoxide production and apoptotic death of the cells. Deletion of the gene presents with hypersensitivity to drugs inhibiting the actin cytoskeleton like wiskostatin and latrunculin B. The growth defect in the presence of these drugs can be mitigated by adding a small non-toxic concentration of hydrogen peroxide, a natural metabolite of superoxide. The mating pheromone, alpha factor, in otherwise wild type cells leads to a large increase in superoxide production which is absent in the yno1 deletion mutants. Isoamyl alcohol, an inducer of pseudohyphae, leads to increased expression of a chromosomally integrated reporter construct consisting of the complete upstream sequence of YNO1 and green fluorescent protein. We hypothesize that Yno1-produced hydrogen peroxide is a natural signaling substance needed to trigger re-organization of the actin cytoskeleton in situations like transition to the stationary phase, reaction to the mating pheromone, alpha factor, and induction of pseudohyphal growth which all need re-structuring of the actin cytoskeleton. Because the standard BY4741 genetic background does not allow pseudohyphae formation, experiments are now under way to test pseudohyphae development in a suitable genetic background by nitrogen starvation, but without isoamyl alcohol.

Among the seven human NADPH oxidases, Nox4 is the one with the highest sequence similarity to the yeast YNO1 gene, is also located in the ER membrane, and produces hydrogen peroxide as a signaling substance. Nox4 is expressed in most human somatic cells. We are showing here (2) that similar to Yno1 of yeast cells, Nox4 in human cells is needed for restructuring of the actin cytoskeleton. Nox4 is strongly expressed in many human tumors. Inhibition of Nox4 in the SH-SY5Y neuronal tumor cell line leads to loss of actin stress fibers and prevents cell migration, which is needed for metastasis. The human Nox4 enzyme is therefore a possible target for cancer therapy.

References:

(1) Rinnerthaler, M, et al. PNAS 109, 8658 – 8663 (2012)

(2) Auer, S., et al. Frontiers in Oncology (2017), accepted with minor revisions

Keywords: *Yeast, Saccharomyces, NADPH, oxidase, YNO1, Cancer*

[S6-2] Duplication of the budding yeast spindle pole body (SPB)

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The spindle pole body (SPB) is the microtubule organizing centre of budding yeast Saccharomyces cerevisiae. Due to the closed mitosis, the SPB is embedded in the nuclear envelope throughout the cell cycle. This enables the SPB to organize nuclear and cytoplasmic microtubules with functions in chromsome segregation and spindle orientation, respectively. Like the mammalian centrosome, the SPB duplicates once per cell cycle using the mother structure as an initiation platform. SPB duplication is initiated in early G1 phase of the cell cycle by the formation of the satellite, a miniature version of the SPB, at the distal end of the bridge. The bridge is an extension of the central plaque and is layered on top of both sides of the nuclear envelope. The cytoplasmic side of the bridge consists of the interacting proteins Sfi1, yeast centrin Cdc31 and the tail-anchored Kar1 protein. At the G1/S phase the satellite grows in size and becomes inserted into the nuclear envelope. Genetic screes identified the SPB insertion network (SPIN) components Bbp1, Mps2, Ndc1 and Nbp1 as SPB-associated factors that are important for the nuclear envelope insertion of the new SPB. In this study we have uncoupled SPB growth from nuclear envelope insertion. This approach revealed several novel findings: 1) The mother SPB can fuse with the satellite if not separated by the bridge structure; 2) Mixing of the central Spc42 SPB layer occurs during SPB fusion in karyogamy; 3) The SPIN components encircle the newly embedded SPB in the nuclear envelope and anchor the SPB in the nuclear membrane; 4) A nuclear pore complex (NPC) becomes recruited to the inserting SPB in G1/S phase. This NPC is important for the insertion of the new SPB into the nuclear envelope. We will discuss models for the role of this NPC in SPB nuclear membrane insertion.

Keywords: *Spindle pole body (SPB), Mitosis, Nuclear pore complex (NPC), Nuclear envelope*

[S6-3] Regulation of mitophagy by (de-)ubiquitylation

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The selective degradation of mitochondria, termed mitophagy, is an evolutionarily conserved process. There is cumulating evidence that this process is linked to numerous human disorders including Morbus Parkinson. One pathogenic mechanism proposed is that the removal of damaged mitochondria is impaired promoting neurodegeneration. Mitophagy can be divided mechanistically into two principal ways: a receptor -mediated and an ubiquitin- mediated pathway. Mutations in genes such as PINK1 or PARKIN that are essential for the ubiquitin-mediated pathway in mammalian cells are known to cause Morbus Parkinson. Recent research suggest that an ubiquitin-mediated mitophagy pathway may also exist in baker's yeast. We addressed the role of all three non-essential ubiquitin genes (UBI1, UBI2, UBI4) in different types of selective autophagy in Saccharomyces cerevisiae. We disect how mitophagy depends on these genes and how this is possibly linked to the deubiquitinase complex Ubp3/Bre5. We further identified an outer membrane protein as a novel player required for mitophagy. In summary, we identified a mitophagy pathway in yeast, which depends on mitochondrial protein ubiquitylation and which is dynamically regulated by ubiquitylation and Ubp3-dependent deubiquitylation.

Keywords: *Parkinson, Mitophagy, Ubiquitinylation, Deubiquitinase, Mitochondria*

[S6-4] A New Factor Involved in the Establishment of the Non-random Inheritance Pattern of the Spindle Pole Bodies in Saccharomyces Cerevisiae

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Many cells, from yeast to higher eukaryotes, divide asymmetrically. During an asymmetric cell division, different polarized factors can segregate preferentially to only one of the two resulting cells, which acts as a mechanism for the generation of cellular diversity. An ideal model to study asymmetric division is the budding yeast Saccharomyces cerevisiae. Interestingly, in this organism the spindle pole bodies (SPBs, the equivalent of the centrosomes in budding yeast) are asymmetrically distributed during cell division, so that they display a non-random inheritance between the mother and bud. Specifically, the old SPB, which is inherited from the previous mitosis, is segregated to the daughter cell, while the new SPB, which is assembled de novo, is retained in the mother cell. Remarkably, asymmetric inheritance of the centrosomes has been also shown in higher eukaryotes, where the age of the centrosome can specify the fate of the cell and the disruption of this inheritance pattern can determine important consequences. We have identified a new factor that is necessary for the establishment of this non-random inheritance pattern of the SPBs between the mother and the daughter cell in budding yeast.

Keywords: *Mitosis, Asymmetric cell division, Spindle pole bodies*

[S7] New biotechnologies

[S7-1] Systems Metabolic engineering of Yeast

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Metabolism represents the core of cellular functions and all cellular processes interact with metabolism through the use of specific metabolites, free energy and/or electron flows. However, metabolism is highly complex involving a large number of chemical reactions, and it is therefore difficult to study metabolism. The many different reactions are traditionally grouped into pathways with dedicated functions, but recent analysis of metabolism has shown that there is a high degree of connectivity between these pathways due to common sharing of co-factors and key metabolites. Also regulation of metabolism is complex due to the requirements for maintaining cellular homeostasis. In this talk I will give illustrations of how different parts of cellular metabolism are connected, i.e. central carbon metabolism, lipid metabolism and protein secretion. I will illustrate how metabolism can be modelled at the genome-scale and how incorporation of protein crowding may be a key determinant for cellular function, and keep proteome homeostasis is an important driver for maintaining proper cellular function. Finally, I will discuss how regulation of metabolism can be studied using different omics analysis, e.g. how we can get new insight into transcriptional regulation through ChIP-exo analysis.

Keywords: *metabolic engineering, systems biology, metabolism*

[S7-2] Engineering enzyme complexes: creating artificial and re-engineering existing enzyme complexes for biotechnological purposes

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The efficiency and flux of metabolic pathways can be hampered by factors like slow diffusion rates, competing pathways or secretion of pathway intermediates. Genetic engineering of new metabolic pathways for biotechnological purposes is often influenced by the same limitations. In nature, such limitations are bypassed by compartimentalization of enzymatic reactions in organelles or multi-enzyme complexes. Various examples of interactions between enzymes catalyzing sequential reactions are known, by which the transfer of reaction intermediates from one active site to the other is accelerated. Direct channeling is achieved within stable multi-enzyme assemblies or by a precise alignment of active centers in a single polypeptide chain. On the other hand, there are examples of enzymes that are co-localized by scaffold proteins, membranes, or cytoskeleton. In such enzyme agglomerates the fast hand-over of intermediates is facilitated by increased local concentrations of reaction partners.

We are developing new concepts for metabolic pathway engineering to assemble enzymes and transporters in protein supercomplexes and to relocate metabolic pathways into synthetic organelles. As an example we will show that by constructing an artificial complex between a sugar transporter and a xylose isomerase in baker's yeast the consumption of xylose was accelerated due to the direct feeding of the enzyme through the transporter. Concomitantly, the production of xylitol as an undesired side-product could be significantly diminished and the production of ethanol could be increased (Thomik et al., Nat Chem Biol, in press).

On the other hand, genetic engineering of existing multi-enzyme complexes is often difficult to achieve due to missing information about the structures, reaction mechanisms and interactions between the individual domains. Nevertheless, we will show how we have recently successfully re-engineered the fatty acid synthase complex of yeast for the production of valuable short- and medium-chain fatty acids, like C8-octanoic acid (Gajewski et al. 2017, Nat Commun 8:14650).

Keywords: *enzyme complex, genetic engineering, xylose, sugar transporter, fatty acid synthase, short-chain fatty acid, octanoic acid, scaffold, substrate channeling*

[S7-3] Biorefine-2G: From Waste Biomass To Biopolymers Using Yeast Cell Factories

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Besides its role as an important model for the study of fundamental cellular processes in eukaryotes, the yeast Saccharomyces cerevisiae is also the organism of choice for many biotechnological applications. Due to its excellent performance in established large-scale processes and well-studied genetics, S. cerevisiae can be modified to host processes leading to conversion of biomass to valuable chemicals as a sustainable alternative to the production of such chemicals from oil. Economic viability of the biorefinery concept of using biomass instead of fossil resources for production of fuels and chemicals needs the development of industrial strains that are sufficiently robust, utilize low value waste biomass streams rich in C5 sugars that are not naturally fermented by S. cerevisiae, and produce added-value chemicals. Here we demonstrate construction of such a yeast cell factory. We present the entire process from the development of advanced molecular tools for engineering of industrial yeast strains, adaptive evolution approaches for generation of platform strains with advanced xylose utilization properties and tolerance to a lignocellulosic biomass feedstock, to construction of strains engineered for production of dicarboxylic acids. These can serve as monomers for development of novel biodegradable polymers. Next generation sequencing analysis of the platform strains performing in xylose-rich media and identification of causative mutations will be presented. Furthermore, relevant metabolic engineering strategies and strain characterization will be shown in

detail. 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).

Keywords: *biotechnology, biorefineries, industrial yeast cell factory*

[S7-4] Deciphering The Roles Of Hybrid And Orphan Genes In The Hybrid Species, Saccharomyces pastorianus

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Saccharomyces pastorianus (lager yeasts) are interspecies hybrids of Saccharomyces cerevisiae and Saccharomyces eubayanus and are classified as Group I and II, based on DNA content and chromosome structure. Recombination events between the parental chromosomes have created a novel set of hybrid chromosomes. Most recombination sites are intragenic, giving rise to a unique set of hybrid genes composed of part S. eubayanus and part S. cerevisiae sequences. In addition to hybrid genes, S. pastorianus encodes for several “orphan” genes, which are conserved in industrial strains of S. cerevisiae only, but for which no known homologues exist in either prokaryotic or eukaryotic species. Such hybrid and orphan genes, if functional, can potentially influence the biochemical, physiological and phenotypic landscape of lager yeasts.

Several hybrid alleles of the gene YGL173C (XRN1) are found in lager yeasts and Group I and II lager yeasts co-express different XRN1 alleles. XRN1 encodes for a 5’ to 3’ exoribonuclease that is required for mRNA degradation in the cell. Given its central role in RNA metabolism, we hypothesise that the co-expression of allelic variants of XRN1 may significantly impact the steady state pool of mRNAs and thus the protein landscape of lager yeasts.

The XRN1 genes, in the same combinations as found in the Group I and II lager yeasts, were cloned into S. cerevisiae Δxrn1. RNAseq data reveals that the co-expression of different allelic variants of XRN1 significantly alters the suite of mRNAs found in the cell. Recapitulating the XRN1 composition of Group I and Group II lager yeasts leads to the differential stabilisation of mRNAs encoding for proteins involved in amino acid metabolism and plasma membrane composition, pathways that significantly influence the fermentation and flavour properties of beer.

A role for the orphan gene “HYPO”, encoding a hypothetical open reading frame, is also being investigated. The expressed protein is located at the plasma membrane where it forms distinct patches. Mass spectrophotometric analysis of HYPO-associated proteins suggests a role for HYPO is stress responses in lager yeasts.

Keywords: *lager yeasts, hybrid chromosomes, hybrid genes, orphan genes, XRN1, RNA metabolism*

[S7-5] Constructing a synthetic Saccharomyces cerevisiae pan-genome neo-chromosome

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The International Synthetic Yeast Genome Project (Sc2.0) is currently in the process of re-engineering and synthesizing the genome of the yeast Saccharomyces cerevisiae. Due to its long-standing use as a key model system, combined with its complete and well-characterized genome sequence, the Sc2.0 consortium is focusing its efforts on the S228c laboratory strain of S. cerevisiae, which has ~6000 genes spread over 16 chromosomes.

However, there are hundreds of different strains of S. cerevisiae and many have distinctive phenotypes that provide an advantage to a specific environmental niche or industry (fermenting wine, leavening bread or brewing beer). These phenotypic differences are the direct result of specific genetic variation between strains and this can range from single nucleotide polymorphisms to the presence of strain-specific genes or gene clusters. The presence or absence of these genes between strains can have striking phenotypic consequences, including providing strains with the ability to synthesize vitamins or to endure specific types of stress or inhibitory compounds.

To provide greater insight into the role of these strain-specific genes, we have identified over 200 kb of non-repetitive DNA, encoding 75 ORFs, which exist across the breadth of strain-specific ORF diversity of the S. cerevisiae pan-genome, but which are absent from the laboratory strain used for Sc2.0. These sequences have been synthesized and assembled, using Sc2.0 principles (watermarking, loxP sites), into a circular, centromeric neo-chromosome. This pan-genome neo-chromosome is now being analyzed to determine the phenotypes that it can impart in the laboratory strain background, while also providing a resource for introducing additional variation into the Sc2.0 genome through processes such as SCRaMbLE.

Keywords: *Saccharomyces cerevisiae pan-genome, Neo-chromosome, Synthetic biology, Synthetic Yeast Genome Project*

[S8] DNA replication, mutation and repair

[S8-1] DNA End Resection and Repair Pathway Choice

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DNA double-strand breaks (DSBs) are cytotoxic lesions that must be accurately repaired to preserve genome integrity. DSBs can be repaired by direct ligation (NHEJ) or undergo 5'-3' resection to generate single-stranded DNA (ssDNA), the substrate for homologous recombination (HR). Once resection initiates, cells are committed to HR. We have previously shown that resection of DNA ends occurs by a two-step mechanism (Mimitou and Symington, 2008). In the first step, the Mre11-Rad50-Xrs2 (MRX) complex and Sae2 incise the 5' strands internal to the DNA ends to form an early intermediate. In a second step, Exo1 or Sgs1 with Dna2 rapidly process the minimally resected intermediates to generate long tracts of ssDNA. In addition to controlling end resection, the MRX complex is required for Tel1 signaling, NHEJ and for end tethering. Xrs2 is essential for nuclear translocation of Mre11, but its role as a component of the complex is not well defined. We found that fusion of a nuclear localization sequence to Mre11 (Mre11-NLS) was able to bypass several functions of Xrs2, including DNA end resection, hairpin resolution and cellular resistance to clastogens; however, NHEJ and Tel1 signaling were not restored indicating a unique function for Xrs2 in these processes (Oh et al, 2016). To further understand the role of Xrs2 in Tel1 signaling, we fused the Tel1 recruitment domain from Xrs2 to Mre11-NLS. The resulting fusion protein was able to bypass the role of Xrs2 in Tel1 recruitment and activation, restoring normal telomere length and Tel1 activation. We will discuss the roles of the MR complex, Xrs2 and Tel1 in DNA repair, HR and suppression of gross chromosome rearrangements.

Keywords: *DNA repair, Homologous recombination, Mre11, Tel1*

[S8-2] DDK-mediated regulation of the deSUMOylating enzyme Ulp2 facilitates early steps of DNA replication

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Dbf4-dependent kinase Cdc7 (DDK) is crucial for chromosome replication initiation, by activating the replicative helicase, MCM. Other means of DDK-dependent control of replication are less understood. Here we uncover that the budding yeast deSUMOylating enzyme, Ulp2, is a DDK substrate that binds to replication origins and promotes early steps of DNA replication. In DDK mutants and phosphodeficient Ulp2 variants, Ulp2 chromatin binding is deregulated, being less concentrated at origins and more diffused along chromosomes. Furthermore, in ulp2 mutants, origin firing-derived replication intermediates and BrdU incorporation efficiency are decreased. Notably, the replication

initiation defects of ulp2 cells were rescued by removing the SUMO-targeted ubiquitin ligase (STUbL), Slx5/8. We propose that DDK regulates Ulp2 distribution and concentration at firing origins to ensure a critical mass of replisome factors poised for replication upon MCM activation.

Keywords: *Dbf4-dependent kinase, DNA replication, replisome factors*

[S8-3] Molecular outcomes of double-strand break repair

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Mitotic recombination between homologous chromosomes leads to the uncovering of recessive alleles through loss of heterozygosity (LOH). Analyses of spontaneous LOH events has suggested that most are initiated by random double-strand breaks (DSBs). To examine this more directly, we used a defined double-strand break to initiate reciprocal LOH between diverged homologs of chromosome IV in Saccharomyces cerevisiae. All LOH events reflected the repair of two broken chromatids, one of which was repaired as a crossover and the other as a noncrossover. Associated gene conversion tracts resulting from the donor-directed repair of mismatches in the heteroduplex DNA formed during strand exchange were mapped using microarrays. Gene conversion tracts associated with individual crossover and noncrossover events were similar in size and position, with half of the tracts being unidirectional and mapping to only a one side of the initiating DSB. Among crossover events, this likely reflected gene conversion on one side of the break, with restoration-type repair occurring on the other side. For noncrossover events, an ectopic system was used to directly compare gene conversion tracts produced in a wild-type strain to unrepaired, heteroduplex DNA tracts that persist in the absence of the Mlh1 mismatch-repair protein. There was a strong bias for unidirectional tracts in the absence of Mlh1 that was lost in strains containing Mlh1, suggesting that MMR acts on heteroduplex DNA that is only transiently present in noncrossover intermediates. Although the molecular features of LOH events generally agreed with those predicted by current recombination models, there were unexpected complexities in associated gene conversion tracts.

Keywords: *mitotic recombination, loss of heterozygosity, double-strand breaks, Saccharomyces cerevisiae, noncrossover, heteroduplex DNA*

[S8-4] Reconstituting chromosome replication

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The process by which active CMG (Cdc45-MCM-GINS) DNA helicase is assembled at eukaryotic replication origins ensures precisely one round of genome duplication in each cell cycle. This process comprises two temporally discrete stages: the minichromosome maintenance (MCM) complex is first loaded as a head-to-head double hexamer (DH) encircling duplex origin DNA during G1 phase; ‘firing factors’ then convert each DH into

two active CMG helicases during S phase. Bidirectional replication requires the loading of two ring-shaped Minichromosome Maintenance (MCM) helicases around DNA in opposite orientations. MCM loading is orchestrated by binding of the Origin Recognition Complex (ORC) to DNA, but how ORC coordinates symmetrical MCM loading is unclear. Using natural budding yeast origins and synthetic sequences, we show that efficient MCM loading requires binding of two ORC molecules to two ORC binding sites. The relative orientation of these sites, but not the distance between them, is critical for MCM loading in vitro and origin function in vivo. We propose quasi-symmetrical loading of individual MCM hexamers by ORC and directed MCM translocation into double hexamers as a unifying mechanism for the establishment of bidirectional replication in Archaea and Eukarya.

In the second stage, the two strands of origin DNA must be unwound and DH remodelled so that individual MCM hexamers each encircle a single DNA strand to seed formation of bidirectional replisomes. We set out to understand the mechanism of this complex second stage using purified budding yeast proteins. We found that that MCM, which hydrolyses ATP during DH formation, remains stably bound to ADP in the DH. Firing factor recruitment triggers MCM to release ADP. Subsequent ATP binding then promotes stable CMG assembly, which is accompanied by untwisting of one helical turn of DNA per CMG and separation of DH into two discrete but inactive CMG helicases. Mcm10, together with ATP hydrolysis, then triggers further DNA untwisting and activation of the helicase. Our experiments elucidate the mechanism of eukaryotic replicative helicase activation, and show that ATP binding plays an analogous role in origin melting in eukaryotes, viruses and bacteria.

Keywords: *DNA replication, biochemistry, cell cycle*

[S9] Yeast pathogens and host interaction

[S9-1] MCC/Eisosome Plasma Membrane Subdomains Promote Stress Resistance and Virulence of Candida albicans

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The plasma membrane is critical for the survival of fungal pathogens in the host because this protective barrier also mediates a wide array of dynamic functions including nutrient uptake, secretion, endocytosis, morphogenesis, cell wall synthesis, and stress resistance. Studies aimed at defining how cells coordinate these diverse functions have shown that the plasma membrane is organized into distinct compartments that vary in size, shape, and stability. Interestingly, a novel type of membrane domain was discovered in yeast that is termed the MCC/eisosome. These domains are distinctive in that they are protein-organized structures that correspond to stable furrows in the plasma membrane. In the human fungal pathogen Candida albicans, mutants that fail to form MCC/eisosomes (pil1 lsp1) display broad abnormalities including defects in cell wall synthesis, polarized morphogenesis, and invasive growth. In addition, MCC/eisosome mutants are more susceptible to a variety of stresses encountered in vivo, such as elevated temperature, oxidation, copper and antifungal drugs. Genetic analysis indicates that proteins recruited to the MCC/eisosomes, such as Sur7, Nce102, Pst1, Pst2, Pst3, and Ycp4, promote the proper regulation of cell wall synthesis and stress responses. Consistent with this, mutants lacking these MCC/eisosome proteins are defective in virulence. Thus, studies on MCC/eisosomes are defining novel plasma membrane functions that will help to identify new targets for antifungal therapy.

Keywords: *Candida albicans, plasma membrane, eisosome, MCC domain*

[S9-2] The Statistical Proteome of Candida Albicans

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The concept of statistical proteins was proposed in 1965 by Carl Woese1. He defined statistical proteins as mixtures of polypeptides whose primary structures are related to some theoretical average primary structure. This concept was overlooked for many years due to its association in the original publication with primordial life forms with poorly defined genetic codes. However, recent studies show that it also applies to existing organisms. We have discovered a statistical proteome in the main human fungal pathogen Candida albicans2. Its genome encodes 6198 protein coding genes (haploid genome) - similar to other fungi -, but ambiguous gene translation by the ribosome diversifies stochastically its proteome producing millions of different proteins that are not degraded by the protein quality control machinery. In other words, in Candida albicans there is no correlation between gene and protein numbers despite the lack of alternative splicing. I

will illustrate in my talk that global re-programming of the genetic code produces statistical proteins that have specific cellular functions. Such proteins increase dramatically phenotypic and genetic diversity, expand adaptation capacity in changing ecological landscapes and influence virulence, biofilm formation and drug resistance.

Keywords: *Candida albicans, protein synthesis, statistical proteins, mistranslation, genetic code*

[S9-3] Comparative Genomic and Transcriptomic Analyses Unveil Novel Features of Azole Resistance and Adaptation to the Human Host in Candida glabrata

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The emergence of azole resistance among Candida glabrata strains is frequent and contributes to increase the incidence of infections caused by this species. In this work we aimed at elucidating the molecular mechanisms underlying resistance to fluconazole and voriconazole in a resistant clinical isolate (FFUL887). Whole-genome sequencing of FFUL887 and subsequent comparison with the genome of the susceptible reference strain CBS138 revealed the existence of prominent differences in several genes documented to promote azole resistance in C. glabrata. Among these was the transcriptional regulator CgPdr1. The CgPdr1 allele encoded by the FFUL887 strain included a K274Q modification not documented in other azole-resistant strains. The significant increase in susceptibility to azoles of the FFUL887 strain upon deletion of the CgPDR1K274Q allele, along with results from transcriptomic profiling rendering evident the upregulation of 80 documented targets of CgPdr1 in the FFUL887 strain, support the idea that K274Q is a novel CgPdr1 gain-of-function mutation. Analysis of the non-coding genome of the FFUL887 and of CBS138 support the idea that in the FFUL887 strain alterations of the CgPdr1-controlled regulatory network may have changed its architecture to improve the expression of azole-resistance genes. Comparison of the genome of the FFUL887 and CBS138 also showed prominent differences in the sequence of adhesin-encoding genes, while comparison of the transcriptome of the two strains showed a significant remodelling of the expression of genes involved in metabolism of carbohydrates, nitrogen and sulphur in the FFUL887

strain; these responses probably reflecting adaptive responses evolved by the clinical strain during colonization of the host.

Keywords: *antifungal resistance, comparative genomics and comparative transcriptomics, CgPdr1 transcription factor, Candida glabrata, fungal infections*

[S9-4] Predator Yeasts: Genomics and Molecular Biology of Necrotrophic Killer Yeasts

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Yeasts of the genus Saccharomycopsis can be isolated from diverse habitats around the globe. They exhibit a unique predacious behaviour, which allows them to feed on and kill suitable fungal prey cells. This is an act of nectrophic mycoparasitism, which is induced under starvation conditions. During predation prey cells are recognized and a penetration peg is formed, with which the prey cell is penetrated and killed. The host range is wide and includes yeasts, such as Saccharomyces cerevisiae and Candida albicans, but also filamentous fungi, e.g. Ashbya gossypii. Starvation of predator yeasts can be induced solely by the lack of methionine in the growth medium. We aim at understanding the biology of predation and characterize molecular pathways and genes required for successful killing of prey cells. To this end we have generated draft genomes of five predator yeasts: Saccharomycopsis fodiens, S. fermentans, S. crataegensis, S. schoenii and Saccharomycopsis spec. The genome sizes range from 12 Mb to 15 Mb. The genome data, which also include several contigs with telomeric repeats, suggest that loss of genes required for sulphate assimilation is causing methionine auxotrophy within Saccharomycopsis species. Interestingly, genomic signatures suggest that Saccharomycopsis species are part of the CTG clade, which reassigned the CTG codon from leucine to serine, e.g. also in C. albicans. This has guided our molecular approach towards tool development for studying predator yeasts. We have developed synthetic markers, e.g. SAK1 providing resistance against the antibiotic G418. Genome profiling indicated the presence of transposons and of gene families encoding proteins that may play a major role for predacious behaviour. This includes genes encoding proteins for cell-cell adhesion, so called flocculins; genes for cell wall degrading enzymes, e.g. chitinases; and proteases. These and other morphogenesis genes required for penetration peg formation offer excellent target genes to analyze predatory behavior in Saccharomycopsis. In order to quantify predation efficiency we have developed a quantitative predation assay. This is based on quantifying CFU of S. cerevisiae grown on a lawn of predator yeast cells.

Keywords: *fungal pathogens, Host-pathogen interaction, Comparative and evolutionary genomics*

[S10] Yeast sociobiology, sensing and signalling

[S10-1] Extracellular Amino Acid-Induced SPS-Sensor Signaling in the Context of Intracellular Metabolic Regulation

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The Ssy1-Ptr3-Ssy5 (SPS) signaling pathway enables Saccharomyces cerevisiae and Candida albicans cells to respond to extracellular amino acids. The pathway controls the activity of two homologous transcription factors Stp1 and Stp2 (Stp1/Stp2). In the absence of inducing amino acids, Stp1/Stp2 are maintained latent due to the presence of N-terminal cytoplasmic retention and promoter exclusion motifs. Signaling events initiated by amino acid binding to Ssy1, the plasma membrane-localized receptor component, activates the Ssy5 endoprotease leading to the cleavage of Stp1/Stp2. The cleaved forms of Stp1/Stp2, lacking their N-termini, target the nucleus where they induce SPS-sensor controlled genes.

We addressed unresolved mechanistic questions regarding the transduction of SPS-sensor derived signals in S. cerevisiae. To test spatial parameters that may affect Stp1 cleavage, we fused Stp1 to the C-terminus of the well-characterized ER membrane-localized chaperone Shr3. Expression of the Shr3-Stp1 chimeric protein suppresses both shr3Δ and stp1Δ null phenotypes, indicating that the chimer retains Shr3 chaperone activity and that Ssy5-catalyzed processing of Stp1 occurs even in an artificial membrane-bound context. Subcellular fractionation indicates that the Shr3-Stp1 fusion exclusively localizes to the ER. Significantly, signaling is unabated in a strain that lacks PM-ER junctions in which Ssy1 and the chimer are spatially clearly separated. Thus, once activated by events linked to the plasma membrane, the catalytically competent Ssy5 Cat-domain can relocate to the ER.

We used Candida albicans to assess how amino acid-induced and SPS-sensor-dependent signals are integrated into the central signaling pathways controlling yeast-to-hyphal morphological transitions in this human fungal pathogen. Our results show that the SPS-sensor dependency is indirect, being the consequence of SPS-sensor induced expression of amino acid permease genes, and that the hyphae-inducing effect of amino acids is derived from their metabolism. Specifically, arginine, ornithine and proline are internalized and metabolized to glutamate in the mitochondria, generating NADH and FADH2. These electron carriers are oxidized by mitochondrial respiration generating ATP, which triggers the Ras1/cAMP/PKA pathway to activate the morphogenic effector transcription factor Efg1. Thus, the SPS-sensor is important to maintain the intracellular amino acid pools that ensure proper metabolic control.

Keywords: *nutrient sensing, signal transduction, regulation of gene expression, intracellular compartmentalization, proteolytic activation latent transcription factors, amino acid pools, mitochondrial respiration, metabolic signaling, morphological switching, Saccharomyces cerevisiae, Candida albicans*

[S10-2] Regulation of TORC1 by Amino Acids: A Central Role for Rag GTPases Within the EGO Complex

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The eukaryotic target of rapamycin complex 1 (TORC1) couples nutrient, energy, and hormonal signals with cell growth, division, and metabolism, and aberrant TORC1 signaling contributes to the progression of human diseases such as cancer and diabetes. Amino acids are important and primeval cues that stimulate TORC1 to promote anabolic processes (such as ribosome biogenesis and protein translation initiation) and inhibit catabolic processes (such as macroautophagy) via the conserved Rag family GTPases. The latter assemble into heterodimeric complexes consisting of Gtr1 and Gtr2 in yeast, or RagA or RagB and RagC or RagD in mammalian cells. These heterodimers are integral to larger complexes coined EGO (exit from rapamycin-induced growth arrest) complex (EGOC) in yeast or Rag-Ragulator complex in mammalian cells, which are predominantly tethered to vacuolar/lysosomal membranes. Because Rag GTPase heterodimers stimulate TORC1 when they contain GTP-loaded RagA/B/Gtr1 and GDP-loaded RagC/D/Gtr2, GTPase activating proteins (GAPs) acting on Gtr1/RagA/B, such as the orthologous yeast SEACIT or mammalian GATOR1 complexes inhibit, while the ones acting on Gtr2/RagC/D, such as the yeast Lst4-Lst7 or the orthologous mammalian FNIP1/2-Folliculin (FLCN) complexes, activate TORC1. The amino-acid sensitive events upstream of GATOR1 that inhibit TORC1 signaling include the cytosolic leucine and arginine sensors Sestrin2 and CASTOR1, respectively. Both sensors stimulate GATOR1 under amino acid deprivation via a poorly understood mechanism involving their binding to the conserved GATOR1-interacting GATOR2 complex coined SEACAT in yeast. How amino acids activate TORC1 through the Lst4-Lst7/FNIP1/2-FLCN GAP complexes is currently not known. In this context, our current research is focused on deciphering the amino-acid sensitive events upstream of the Rag GTPase regulators in yeast, which likely involve both vacuolar and cytoplasmic amino acid sensors. Due to the evolutionary conservation of the EGOC and its regulators, our studies in yeast are expected to contribute to the understanding of the molecular mechanisms leading to diseases that are associated with hyperactive mammalian TORC1.

Keywords: *TORC1, Rag GTPases, growth control, amino acid signalling*

[S10-3] A rudimentary apoptotic pathway protects sporulating yeast from lethal viral accumulation

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As Programmed Cell Death (PCD) acts in opposition to the fitness of the cell executing it, how PCD came about during evolution remains a quandary. A logical hypothesis is that cells capacities for self-killing must have co-opted processes that originally promoted cell survival. We have discovered what might be regarded as a living fossil of PCD that illuminates this hypothesis. Yeast sporulation involves a 5-way cell division, with the 4

meiotic products developing within the remnant of the cell that produced them. We showed that PCD of this remnant occurs as an intrinsic aspect of yeast gametogenesis and is executed through developmentally programmed vacuolar rupture. Intriguingly, undeveloped meiotic nuclei that are frequently swept up in this PCD are subjected to fragmentation of their genomic DNA into nucleosomal ladders, a hallmark of apoptosis. This phenomenon is dependent on NUC1, the yeast homolog of mitochondrial endonuclease G, and is associated with mitochondrial membrane depolarization in the remnant cell that occurs prior to vacuolar rupture. Despite being a prominent feature of apoptosis, the raison d'être for genome fragmentation during mammalian apoptosis remains unknown. Similarly, the underlying role of this DNA fragmentation pathway during yeast meiotic PCD has remained obscure. Yeast possesses numerous viral entities whose persistence requires successful meiotic transmission. We have now determined that NUC1 functions in a rudimentary apoptotic pathway that promotes yeast survival through an innate immune defense against these viral entities. Accumulating L-A and "Killer" dsRNA viruses that are endemic to yeast triggers the release of Nuc1 through an apparent MOMP-like (mitochondrial outer membrane permeabilization) phenomenon, resulting in their downregulation. Failure of Nuc1 to curb the accumulation of the Killer virus causes lethality in mitotic cells. Molecular, biochemical, and genetic analysis confirms that accumulating Killer virus within the cells causes this lethality, illuminating that this rudimentary apoptotic pathway therefore functions in an innate immune capacity. Extension of these findings to meiotic cells confirms that NUC1 prevents the hyper-accumulation of Killer toxin following meiosis. We are interested in the roles this pathway may play for defense of the yeast germline against other genetic parasites such as retrotrasnposons.

Keywords: *Yeast viruses, Sporulation, Endonuclease G, Programmed Cell Death, Evolution, Mitochondria*

[S10-4] The Hog1p kinase regulates the Aft1p transcription factor to control iron accumulation

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Iron acquisition systems have to be tightly regulated to assure a continuous supply of iron, since it is essential for survival, but simultaneously to prevent iron overload that is toxic to the cells. Our previous work revealed that cells lacking the sphingomyelinase Isc1p exhibit an upregulation of genes involved in iron uptake leading to increased levels of iron (1). In this study, we show that isc1Δ cells also exhibit deregulated iron localization with a decrease in the vacuolar levels. In isc1Δ cells, despite the presence of iron, the low-iron sensing transcription factor Aft1p is dephosphorylated, accumulates in the nucleus and is transcriptionally more active indicating that Aft1p is improper activated. Aft1p activation underlies iron accumulation in isc1Δ cells since deletion of AFT1, or expression of an Aft1p phosphomimetic mutant S210DS224D that favours its nuclear export, abolished iron

accumulation. We also show that Aft1p is dephosphorylated and activated under iron replete conditions in cells lacking Hog1p, a kinase with altered activity in isc1Δ cells (2). Co-immunoprecipitation analysis and an in vitro kinase assay revealed that Hog1p interacts with and directly phosphorylates Aft1p. Moreover, Hog1p-Aft1p interaction decreased in isc1Δ cells. We propose that the decrease in Aft1p-Hog1p interaction leads to Aft1p dephosphorylation in isc1Δ cells and, as consequence, to Aft1p activation and iron overload.

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1 Almeida T et al. (2008) Mol Biol Cell 19, 865-76

2 Barbosa AD et al. (2012) Mech Ageing Dev 133, 317-30

Keywords: *Iron, Isc1p, Aft1p, Hog1p, Cell signalling*

Workshops

[W1] Yeast population, comparative and evolutionary genomics

[W1-1] Understanding Adaptation and Fitness Trade-offs in Yeast

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The study of how adaptive mutations affect fitness in their evolution environment and trade-off in alternative environments is essential for understanding the evolutionary process. However, it is only recently that advances in sequencing technology have provided an unprecedented opportunity to study the genetic and physiological basis of adaptation and fitness trade-offs at the molecular level.

In our previous work, we developed a DNA barcode-lineage system in yeast to track their evolutionary dynamics under a glucose-limited serial transfer condition. This system enabled further isolation of thousands of independently evolved lineages from the same experiment and identification of their adaptation-driving mutations.

In this work, I have developed a high-throughput approach to investigate the physiological basis of adaptation and trade-offs in these evolved lineages, by decomposing their fitness into distinct physiological phases in a highly quantitative manner. To achieve this, I measured the fitness of thousands of evolved lineages in bulk under experimental conditions modified from the Evolving Condition (EC, which includes lag, fermentation and respiration phases, but not stationary phase), where the lengths of fermentation, respiration, and stationary phases were systematically varied. Qualitatively, I discovered that self-diploidized adaptive lineages (diploids) and adaptive haploids differed in their adaptive strategies: diploids only gained fitness during fermentation; however, adaptive haploids gained fitness from both fermentation and respiration. Quantitatively, while all adaptive lineages had similar fitness changes during fermentation, their fitness changes during respiration were determined by their genetic basis and were strongly correlated with their fitness under the EC. Moreover, I observed an anti-correlation between respiratory fitness change and stationary fitness change, indicating an intrinsic trade-off between respiratory growth and survivability during stationary phase. Lastly, since mutants responded to different conditions in quantitatively distinct ways, I was able to predict genotypes from their collective set of fitness measurements.

Our study demonstrates that fitness measurements under rationally-designed conditions are a powerful way to study the physiological basis underlying adaptation and trade-offs,

and to cluster genetically/physiologically similar lineages without detailed molecular analysis.

Keywords: *Adaptation, Serial transfer, High-throughput fitness decomposition, Fitness trade-off, Physiology*

[W1-2] The Genetic Basis for Gamete Inviability

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Sexually reproducing organisms are dependent on the production of gametes for the continuation of their genetic lineage. Therefore, the ability to undergo a successful meiosis, producing viable and fully functional gametes is critical; failure to do so may result in weak or inviable offspring and the end of the lineage. Compounding on the difficulty to pass alleles on to the next generation, interactions between different alleles may also result in sub-optimal gametes. To investigate the underlying genetics behind why gametes are inviable we have constructed six hybrids spawning from crosses between highly diverged representatives of four Saccharomyces cerevisiae lineages. We recently published reference quality genome assemblies for the four parents and these end-to-end assemblies give us a thorough understanding of all the genetic differences in the hybrids, from single nucleotide polymorphisms to structural variation. Thanks to this, we are in a position to accurately describe how gamete viability in a hybrid is dependent on the genetic makeup of the parents. By dissecting and whole genome sequencing 2,500 gametes from each of the six hybrids, we are producing a resource of 15,000 gametes with varying viability and fitness. Using the sequence data we are exploring the impact of the recombination landscape, aneuploidies and genetic interactions on gamete inviability, and relating these phenomena to underlying genomic differences between the parents. Numbers and types of aneuploidies varied across gametes depending on parent combinations and genetic distance between parents. Aneuploidies correlate well with the gamete inviability but the majority of inviable gametes are not explained by this. We are currently exploring the effect of the recombination landscape on gamete viability and fitness, and investigating the role of allele-allele interactions.

Keywords: *Gamete inviability, fitness, genomic variation, meiotic recombination, aneuploidies*

[W1-3] Towards a Species-wide View of the Genetic Architecture of Traits

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One of the main goals in modern genetic is to understand the relationship existing between genotype and phenotype. However, it is still difficult to precisely address molecular bases underlying complex traits. Are all the inherited traits mainly governed by additivity? What is the degree of non-additivity and how do they affect trait heritability in a population? A better understanding of the genetic architecture of complex traits requires both a precise estimation of genetic components governing phenotype and the completion of a study on a broad panel of individuals and traits, representing the totality of the species diversity.

To obtain this unbiased view of traits genetic architecture, the yeast Saccharomyces cerevisiae represents a powerful and unique model with its ease of use and the number of tools available. With the recent resequencing of more than 1,000 complete genomes of natural isolates in our lab, this dataset gives us the most complete view of the phenotypic and genetic diversity of a eukaryotic model.

Digging into yeast genetic architecture of trait has several targets. First, we performed a breakdown of the genetic components responsible for the phenotypic variation. In a second time, a precise estimation of the expressivity of traits on different genetic backgrounds has been be carried out. Finally this work allowed to characterize both rare and common variants associated with specific traits.

We are currently focusing on the genetic architecture of traits in hybrid cells. To do so, we generated a diallel cross panel of 3,025 hybrids resulting from the systematic intercrossing of 55 haploid strains available in both mating type. Phenotyping of these hybrids has been carried out on 53 stress related traits (20 compounds with different concentrations) giving 160 325 cross/trait combination.

To estimate the proportion of additivity versus non-additivity in phenotypes and to obtain a view on the importance of dominance, we looked at heterosis depicting the phenotypic shift of an hybrid compared to the mean of its two parents. Diallel panel offers a good opportunity to evaluate the genetic variance components in hybrids using combining abilities which also grants an estimation of broad and narrow-sense heritability.

The last step on the diallel hybrid panel analysis has been to perform genome-wide association studies (GWAS) to uncover common additive variants responsible for particular phenotypes.

Keywords: *Genetic Architecture of Traits, Non-additivity, Heterosis, Diallel Cross, Combining abilities, Genome-wide Association Study*

[W1-4] Natural Genetic Variation in Saccharomyces cerevisiae Reveals Diet-Dependent Mechanisms of Chronological Life Span Control

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University of Luxembourg (Luxembourg)

Aging is one of the most fundamental processes of life. As it is a leading risk factor for many common diseases in humans, research on the molecular mechanisms involved in this process is expected to lead to a better understanding of age-associated diseases and to

promote the development of health and/or life span extending strategies. Although aging-related mechanisms have widely been investigated using deletion collections isogenic the reference strain of Saccharomyces cerevisiae, natural isolates have been poorly exploited to address this research challenge. So far, the vast majority of age related studies have been performed in glucose-limited conditions. The fact that human beings are mostly living in rich-diet conditions, might limit the transferability of the findings in yeast. To investigate the impact of both genetic variability and growth condition on life span regulation, we used a collection of natural isolates of S. cerevisiae aged under various environmental conditions. Through the analysis of a segregating population, we found that genes governing the regulation of life span are highly dependent on the glucose concentration. While we identified the RIM15 gene, encoding a protein kinase, to modulate life span in calorie-restricted conditions, we could not detect its regulatory effect in rich-diet condition, where instead a single mutation in the SER1 gene increases the longevity. By comparative studies of different sugars, we determined the carbon source to be crucial for life span modulation. We found that the use of the trisaccharide raffinose (Galactose-Glucose-Fructose) as sole carbon source increases life span to the same extend as calorie restriction. We notably identified that the presence of members of the MEL gene family is involved in this increase. These MEL genes are usually found in the subtelomeric regions of few strains only, and encode enzymes able to cut the bound between galactose and glucose in the raffinose sugar. Using a targeted metabolomics approach, we validated that strain containing a MEL gene can use galactose, glucose and fructose coming from raffinose whereas strains lacking such gene will be restricted to the use of fructose as carbon source, while the galactose-glucose disaccharide remains in the medium. Taken together, the data proves that aging studies in natural yeast variants under different environmental conditions open new avenues for deciphering the genetic and molecular basis of life span control.

Keywords: *Population genomics, Linkage mapping, Aging mechanisms*

[W1-5] Cooperation and division of labour in Saccharomyces cerevisiae

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Division of labour between different specialized cell types is a hallmark of biological complexity in multicellular organisms. However, it is increasingly being recognised that division of labour also plays an important role in the lives of predominantly unicellular organisms. Saccharomyces cerevisiae displays several phenotypes that could be considered a division of labour, including quiescence, apoptosis and biofilm formation, but they have not been explicitly treated as such. We discuss each of these examples, using a definition of division of labour that involves phenotypic variation between individuals within a population, cooperation between individuals performing different tasks, and maximisation of the inclusive fitness of all individuals involved. We then propose future research directions and possible experimental tests using S. cerevisiae as a model organism for understanding the very first stages of the evolution of division of labour.

Keywords: *sociomicrobiology, cell types, altruism, differentiation, cooperation*

[W1-6] Adaptation of Yeast to Anthropogenic eEnvironments Using Comparative Genomics

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The diversity of alcoholic beverages developed by Man around the world is a remarkable outcome of history. These fermented beverages rely on the same unicellular fungi: Saccharomyces cerevisiae, which is also found in other fermented foods and natural resources. The development of these fermented beverages has shaped the diversity of this yeast species (Legras et al. 2007).

Using genomic data from 82 individuals from these various environments (biological ageing, wine fermentation, sugar cane fermentation, fermented milk, oak...), we could show that S. cerevisiae strains have gained traits reflecting the constraints imposed by natural or artificial selection in several anthropogenic ecological niches. Our results indicate that the improvement of various traits have been achieved in the different populations through different mechanism: mutation, hybridization with other yeast species or horizontal gene transfer from other yeast genera, and left specific signatures in their genomes leading to modern domesticated lineages such as Flor strains (Coi et al 2017). We present here multiple clusters of genes transferred horizontally from distant genus, genes showing evidence of positive selection and genomic regions with signature of selective sweeps specific to the different populations. In several cases we could associate this genetic features to specific phenotypes traits such as for flor or cheese strains.

Legras J-L, et al. (2007). Molecular ecology, 16, 2091–2102.

Coi AL, et al. (2017) Molecular Ecology, 38, 42–49.

Keywords: *Saccharomyces cerevisiae, Population genomics, adaptation*

[W1-7] Yeast population genomics reveal origin and evolution of a classic model organism

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The baker’s yeast S. cerevisiae has had a long association with human activity, leading to the idea that its use in fermentation leads to its domestication. However, recent studies

revealed that S. cerevisiae has a rather ubiquitous distribution in the wild, not limited to human-associated environments, showing that its history goes far beyond its association with humans.

In the past decade, we applied population level sequencing to thousands of S. cerevisiae strains to further illuminate the population structure and the impact of human activity. Many breeds associated to specific human process have specific genomic signatures likely driven by adaptation to industrial environments. In parallel, genome analysis of highly diverged wild lineages that predate domestication is consistent with south East Asia as the geographic origin of S. cerevisiae and support a single out-of-China origin followed by several independent domestication events. While domesticated isolates diverge by genome content, ploidy and aneuploidy variation, wild isolate genome evolution is mainly driven by the accumulation of single nucleotide variants.

Furthermore, we use long-read sequencing to generate end-to-end genome assemblies for strains representing major subpopulations of S. cerevisiae and its wild relative S. paradoxus. These population-level high-quality genomes with comprehensive annotation enable precise definition of chromosomal boundaries between cores and subtelomeres and a high-resolution view of evolutionary genome dynamics. We observed contrasting evolutionary dynamics across the genomic landscape between S. cerevisiae and S. paradoxus. Taken together, many of these observed differences probably reflect the influence of human activities, which sheds new light on why S. cerevisiae, but not its wild relative, is one of our most biotechnologically important organisms.

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Keywords: *population genomics, domestication, structural variation*

[W1-8] Mechanism and Impact of De Novo Gene Emergence Across 15 Yeast Genomes

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How new genes and new protein functions arise is a fundamental question in evolution. The creation of novel functions using existing genes as raw material (gene duplication, gene fusion etc.), once considered the only plausible path to innovation, has been extensively studied. Nonetheless, the discovery of de novo gene emergence from previously noncoding sequences proved that novel genes can also evolve "from scratch". A decade after the first de novo genes were found in Drosophila, the underlying molecular mechanisms as well as the overall impact of de novo emergence on genome evolution are still a matter of debate.

We developed a comprehensive methodology combining extensive homology searches, protein evolution simulations and machine learning to, for the first time, reliably identify de novo protein-coding genes across multiple genomes. We applied our method on 15 genomes from the 2 densely sampled yeast genera, Lachancea and Saccharomyces sensu stricto, and complemented the in silico analyses with Mass Spectrometry proteomics experiments. We found that the rate of gene emergence is steady within each genus but varies 10-fold between the 2 genera. Our results suggest that de novo genes have originated from noncoding regions that are significantly more GC-rich compared to the intergenic genome average. De novo genes were predominantly found divergently oriented relative to their neighbours, suggesting that their emergence was likely driven by divergent transcription from bidirectional promoters. In Saccharomyces, where data were available, de novo genes were found associated to recombination hotspots. These findings lead us to propose that in yeasts, the combination of 1) high GC-content sequences around bidirectional promoters, a result of GC-biased gene conversion following Double Strand Breaks, and 2) constant divergent transcription of these same regions, provide conditions that favor de novo gene emergence by generating transcripts with lower probability for AT-rich stop codons and thus higher probability for ORF formation. High GC-content in these transcripts could also lead to higher translational efficiency which would further increase their protein-coding potential.

Keywords: *de novo gene emergence, novel genes, evolutionary innovation, yeast genome dynamics, Taxonomically Restricted Genes, Orphan genes*

[W1-9] Environmental long-term Adaptation of Pichia pastoris – Universal Strategies and species-specific Traits of an industrial Yeast Species.

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Experimental evolution has been primarily applied in classical model organisms such as Escherichia coli and Saccharomyces cerevisiae. Thus, whereas the benefit of this approach has been evaluated for biotechnological applications of these species, little is known for non-conventional but biotechnologically important yeast species. Towards this end, we established a first comprehensive adaptive landscape of the methylotrophic yeast Pichia pastoris, which is applied in recombinant protein production processes. Environmental

adaptation was performed by serial transfers in various growth environments and for several hundred generations, followed by growth profiling and genome sequencing. Several nutrient-rich and nutrient-poor growth environments with glucose and/or methanol as carbon sources were used for the adaptation of wildtype and glycosylation-deficient OCH1 knockout populations. Generally, adaptation was obvious as evolved populations showed increased growth rates or improved competitive fitness in adaptive conditions, whereas varying degrees of trade-offs were observed in non-evolutionary growth conditions. On the genome level, several environment-specific recurring mutational hotspots were observed among 55 evolved P. pastoris clones. For instance, multiple clones from independently methanol-evolved populations showed mutations of the alcohol oxidase 1 (AOX1) gene, leading to reduced AOX activity despite increased growth rates. Furthermore, methanol- and glucose-selection lead to multiple independent clones with mutations of two hitherto uncharacterized P. pastoris-specific transcription factors involved in environmental control. Another major mutational target was identified in the high osmolarity glycerol (HOG) signaling pathway. Strikingly, we observed HOG signaling pathway mutations in a genotype- and environment dependent manner as they emerged in opposing environmental conditions in wildtype and OCH1 populations. In this context, our data indicated a generally reduced adaptive potential, as well as diverging adaptive trajectories of glycosylation-deficient P. pastoris populations. Altogether, we provide evidence for highly species-and genotype-specific mutations, which improves our understanding of the regulatory features of wildtype and mutant strains of biotechnologically relevant yeast species and also highlight the potential of this experimental approach for recombinant protein production processes.

Keywords: *Pichia pastoris, environmental stress, OCH1, experimental evolution, genome sequencing*

[W1-10] The evolution of the temporal program of genome replication

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DNA replication plays a major role in genome evolution by directly or indirectly promoting the formation of point mutations and large-scale chromosomal rearrangements. Conversely, both small and large-scale mutational events could possibly impact the evolution of the temporal program of genome replication itself. However, the evolutionary dynamics of the replication programs and the mechanisms by which the cohort of active replication origins is progressively renewed during evolution remain unclear. We experimentally determined the temporal program of genome replication in 10 related Lachancea species and characterized the evolution of their replication programs and origin usage at the genus level. We constructed families of orthologous replication origin to reconstruct the evolutionary scenario of origin gains and losses in the Lachancea phylogenetic tree since the species diverged from their last common ancestor. We inferred the functional properties of all replication origins, such as their respective chromosomal location, firing time and efficiency, and revealed rules that govern the birth and death, or conservation, of active replication origin over evolutionary time.

Keywords: *Lachancea, Replication, Replication program, Replication origin, Genome, Evolution, comparative genomics, functional genomics*

[W2] Modern yeast biotechnology

[W2-1] Yeast Biotechnology in the 21st Century

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It is no overstatement that yeast has shaped the development of modern biotechnology more than any other microorganism. Brewing technology and bakers' yeast production, both intertwined over history, marked the initial cornerstone of the development of current bioprocesses for production of bioethanol, amino acids and organic acids, to biopharmaceutical proteins made with mammalian cells. Recent developments of systems and synthetic biology enabled the expansion of the product range made by yeasts to non-native primary metabolites, complex heterologous secondary metabolites and diverse proteins. In the last decades the pool of yeast species employed in biotechnology has been widely expanded, and a tremendous amount of knowledge has been accumulated to characterize these yeasts.

To introduce the Workshop on Modern Yeast Biotechnology, current developments will be highlighted based on recent literature and research in our laboratory on the pentose utilizing yeast Sugiyamaella lignohabitans and the methylotrophic yeast Komagataella phaffii (Pichia pastoris).

Keywords: *yeast biotechnology, metabolism, pentose utilization, methylotrophy*

[W2-2] Ethyl acetate production by the elusive alcohol acetyltransferase from yeast

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Ethyl acetate is an industrially relevant ester that is currently produced exclusively through unsustainable processes. Many yeasts are able to produce ethyl acetate, but the main responsible enzyme has remained elusive, hampering the engineering of novel production strains. Here we describe the discovery of a new enzyme (Eat1) from the yeast Wickerhamomyces anomalus that resulted in high ethyl acetate production when expressed in Saccharomyces cerevisiae and Escherichia coli. Purified Eat1 showed alcohol acetyltransferase activity with ethanol and acetyl-CoA. Homologs of eat1 are responsible for most ethyl acetate synthesis in known ethyl acetate-producing yeasts, including S. cerevisiae, and are only distantly related to known alcohol acetyltransferases. Eat1 is therefore proposed to compose a novel alcohol acetyltransferase family within the α/β hydrolase superfamily. The discovery of this novel enzyme family is a crucial step towards the development of biobased ethyl acetate production and will also help in selecting improved S. cerevisiae brewing strains.

Keywords: *Ethyl acetate, yeast, alcohol acetyltransferase, Saccharomyces cerevisiae, α/β hydrolase, Escherichia coli*

[W2-3] Polygenic Analysis of High Acetic Acid Accumulation, a Novel Putative Probiotic Property of Saccharomyces cerevisiae var. boulardii

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Probiotics are microorganisms that confer beneficial properties on their mammalian host when ingested. Saccharomyces boulardii is the best established yeast probiotic1. Since it was isolated from lychee fruit by Henri Boulard in 1920, it has been used as a preventive and therapeutic agent against diarrhea. Numerous positive health effects have been attributed to its use. These include increasing the availability of nutrients in fermented food, degradation of pathogenic toxins and support to the immune system. It also increases short-chain fatty acid levels in the gut, with possible antibacterial action2. Upon investigation of a collection of S. boulardii strains, we noticed for some strains a large zone of bacterial growth inhibition in an agar-well diffusion assay with an E. coli indicator strain exposed to the cell-free supernatant of an S. boulardii culture. Investigation of these supernatants showed that they contained a high concentration of acetic acid. We performed quantitative trait locus (QTL) mapping by pooled-segregant whole-genome sequence analysis of S. boulardii acetic acid accumulation capacity. For that purpose, a diploid S. boulardii was made homozygous for mating type, crossed with a compatible S. cerevisiae diploid strain, and after two rounds of sporulation, a haploid segregant with a comparable acetic acid accumulation as the parental S. boulardii strain was isolated. It was then crossed with a haploid S288c strain. The hybrid was sporulated and 549 segregants were evaluated for acetic acid accumulation, after which 32 superior segregants were pooled and sequenced. Mapping of the SNP variant frequency revealed two major and some minor QTLs. They were analyzed by reciprocal hemizygosity analysis and for each major QTL a causative gene for acetate accumulation was identified. Although reciprocal allele exchange between S. boulardii and S. cerevisiae confirmed the causative character, additional important causative genes in minor QTLs appear to be present. This is currently being investigated in more detail. Overall, this methodology can be applied to investigate other traits linked to the probiotic action of S. boulardii.

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Keywords: *Polygenic analysis, Probiotic, Saccharomyces cerevisiae var. boulardii, High acetic acid accumulation*

[W2-4] Saccharomyces Cerevisiae as a Production Platform for Short-/Medium-chain Fatty Acid-derived Alcohols and Alka(e)nes

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Due to the limited nature of fossil fuel resources the demand for renewable bio-based chemicals and advanced biofuels will increase steadily. Fatty acids are considered as strategically important platform compounds and can suit as precursors for biofuels. Since mainly the chain length of hydrocarbons determines their physico-chemical properties regarding combustion processes in engines, this is an important parameter. Here we will present the engineering of yeast for the production of specific short-/medium-chain fatty acid derivatives (C6-C12), such as fatty alcohols and fatty alka(e)nes which share substantial characteristics with currently used fossil fuels and give access to new drop-in biofuels without the need of engine or infrastructure adaption.

The yeast S. cerevisiae does not naturally accumulate short-/medium chain fatty acids. Therefore, metabolic engineering of the fatty acid synthetase (FAS) via rational mutagenesis to specifically produce short-chain fatty acids was done. Mutating one specific amino acid in the FAS complex (R1834K) enables S. cerevisiae to produce a high yield of octanoic acid (Nat. Commun. doi:10.1038/NCOMMS14650), a fatty acid with a length of eight carbon atoms. Based on octanoic acid it is necessary to engineer synthetic pathways specifically for the biosynthesis of short-/medium-chain fatty alcohols and alka(e)nes. One route is the conversion of octanoic acid to its corresponding aldehyde by the carboxylic acid reductase (CAR) from Mycobacterium marinum. This fatty aldehyde can then be reduced to 1-octanol by endogenous alcohol dehydrogenases (ADHs). 1-octanol has been identified as an attractive target compound with diesel-like properties. A big challenge for engineering such pathways is to achieve high yields and titers due to the low enzymatic activity of most enzymes, the toxicity of some target compounds, secretion of intermediates or production of undesired byproducts. Here, we will present the synthesis of 1-octanol with yeast and strategies to enhance its production.

Keywords: *Biofuel, Fatty acid biosynthesis, Fatty alcohol, Fatty alkanes/alkenes, Saccharomyces cerevisiae*

[W2-5] Construction of the flavinogenic yeast Candida famata overproducing riboflavin, flavin nucleotides and bacterial fravin antibiotics aminoriboflavin and roseoflavin

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Flavinogenic yeasts overproduce riboflavin under iron limitation. Candida famata belongs to this group and is one of the most flavinogenic yeasts known. Earlier, this species was

used for construction of the industrial riboflavin producers; however, they appeared to be unstable so corresponding process was shut down several years ago. Recently, we have constructed stable riboflavin overproducer of C. famata using combined approaches of metabolic engineering and classical selection. This was achieved due to overexpression of the gene SEF1 coding for transcription activator of the structural genes of riboflavin pathway, gene IMH3 coding for IMP dehydrogenase and selection for resistance to several antimetabolites, mainly structural analogs of purines and riboflavin. Riboflavin overproducer was used for construction of the strains overproducing flavin nucleotides FMN and FAD due to overexpression of the genes FMN1 and FAD1 coding for riboflavin kinase and FAD synthetase, respectively. There is known the antibiotic of flavin nature, roseoflavin, produced by the soil actinomycete Streptomyces davawensis. Roseoflavin is effective against pathogenic Gram-positive bacteria including methicillin-resistant Staphylococcus aureus. It was found recently that roseoflavin is synthesized from FMN through aminoriboflavin. We decided to construct yeast C. famata producing aminoriboflavin and roseoflavin. For this, C. famata gene FMN1 and S. davawensis genes rosB and rosA were overexpressed under control of strong constitutive TEF1 or inducible MAL2 promoters. Strains of C. famata with overexpression of the own FMN1 gene and the heterologous rosB gene accumulated aminoriboflavin (biosynthetic precursor of roseoflavin). Strains which co-overexpress FMN1, rosB and rosA genes have also been constructed. Apparently this is the first evidence for successful construction of the yeast strains producing bacterial antibiotici. Perspectives of the construction of the competitive producers of riboflavin, flavin nucleotides and flavin antibiotics are discussed.

Keywords: *flavinogenic yeast, Candida famata, riboflavin, flavin coenzymes, FMN, FAD, flavin antibiotics, roseoflavin, aminoriboflavin, metabolic engineering*

[W2-6] Association between Alcoholic Fermentation and S-adenosylmethionine in Beer Brewing

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In lager beer brewing, the fermentation performance of the bottom-fermenting brewer's yeast Saccharomyces pastorianus impacts beer quality. In recent years, strains suited to high-gravity brewing have been favored because they reduce costs and energy as well as permit the production of new types of beer such as those with high alcohol content. Conditions of high gravity, high osmotic pressure, and high alcohol concentration are stressful for brewer's yeast, resulting in a fermentation delay during high-gravity brewing. Thus, the breeding or selection of yeast with increased fermentation rates in high-gravity wort is highly desirable. We sought to identify factors that govern the fermentation ability of such strains. We focused on a sake yeast strain of Saccharomyces cerevisiae that is known to yield higher levels of alcohol (≧20% (v/v)) during sake fermentation. Recent studies showed that a defective transition to the G0 phase is one reason why sake yeast produce high concentrations of alcohol (Watanabe et al., J. Biosci. Bioeng. 2011; Watanabe et al., Appl. Environ. Microbiol. 2012; Watanabe et al., Appl. Environ. Microbiol. 2016). We

previously constructed recombinant strains of bottom-fermenting brewer's yeast with improved fermentation rates of by applying knowledge gained from sake yeast (Oomuro et al., J. Biosci. Bioeng. 2016). However, beers brewed using recombinant strains are disfavored in the Japanese market; beers brewed with traditionally bred (non-genetically modified) yeast would be more appealing. Here, we describe work focused on fermentation metabolites of yeast. Recent studies of sake yeast revealed the accumulation of high levels of S-adenosylmethionine (SAM) (Kanai et al., J. Biosci. Bioeng. 2017) in sake yeast cells. Other work has demonstrated that SAM is involved in regulating glycolysis (Li et al., Mol. Cell 2015). We therefore focused on the relationship between SAM levels and alcohol fermentation by bottom-fermenting brewer's yeast. High-gravity fermentation of SAM-supplemented wort, or the use of yeast able to accumulate high levels of SAM, provided increased fermentation rates compared to those of controls. These results suggested that intracellular SAM accumulation is associated with enhanced fermentation by bottom-fermenting brewer's yeast. Thus, breeding for increased intracellular SAM accumulation in brewer's yeast may facilitate the engineering of strains better suited to high-gravity brewing.

Keywords: *Alcoholic fermentation, S-adenosylmethionine, Saccharomyces pastorianus, ADO1*

[W2-7] Enhancing Heterologous Protein Secretion in Cellulolytic Saccharomyces cerevisiae Strains

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A major obstacle to using the yeast Saccharomyces cerevisiae in single-step hydrolysis and fermentation of cellulosic material for second generation bio-ethanol production is its inferior yields of secreted heterologous cellulases. We have attempted to enhance heterologous protein secretion through rational design strategies involving several proteins integral to the secretion pathway. 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. We have demonstrated an increase in secretory titers for the Talaromyces emersonii Cel7A (a cellobiohydrolase) and Saccharomycopsis fibuligera Cel3A (a β-glucosidase) expressed in Saccharomyces cerevisiae through single and co-overexpression of some of the "ER-to-Golgi" and "exocytic" SNARE components. We conclude that SNARE proteins fulfil an essential role within a larger cascade of secretory machinery components that could contribute significantly to future improvements to S. cerevisiae as protein production host. As heterologous protein secretion was previously shown to induce cellular stress in yeast, we also investigated the role of stress response genes in successful heterologous protein production. We have demonstrated that overproduction of certain stress related enzymes could enhance heterologous cellulase production by over 2-fold. Finally we will report how a strain breeding approach was used to generate yeast strains for improved cellulase secretion. These results demonstrate the unexploited potential of S. cerevisiae as heterologous protein production host.

Keywords: *cellulosic ethanol, consolidated bioprocessing, enzymatic hydrolysis, yeast secretion pathway, cellobiohydrolase expression, rational strain design*

[W2-8] Biodiversity-based Sources for Industrial Yeast Strains

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Industrial yeast strains require a set of traits – from altered metabolic fluxes to increased robustness – that are most often polygenic and as such rather difficult to engineer on the genomic level. Recent development of novel tools for analysis of polygenic traits in Saccharomyces cerevisiae and genetic characterization of a large number of its natural strains made it possible to relatively easily identify causal genes or alleles for biotechnologically relevant traits. These can then be transferred from strains possessing such traits to industrial strains which do not. We have applied such an approach to determine the genetic architecture of neutral lipid accumulation in S. cerevisiae and have been investigating the effect of the transfer of causal alleles between strains with initially different neutral lipid content. Our results show that, also for this non-selectable trait, the approach can result in accurate identification of causal alleles and in predictable effects of genetic manipulations of potential industrial yeast strains.

Apart from the within-species genetic diversity, also genes from even other domains of life can be very useful in the development of industrial yeast strains. One such example is utilization of genes encoding bacterial glycosidases for yeast strains developed for consolidated bioprocessing. To functionally assess potentially useful new enzymes, we have combined with yeast display a metagenomics approach that identified genes for glycosidases from ruminal bacteria evolved for specific biomass sources. Both approaches for tapping biodiversity-based sources discussed here are complementary and should enable advancement of development of new industrial yeast strains.

Keywords: *industrial yeast strains, polygenic traits, biodiversity, consolidated bioprocessing*

[W3] Controlling gene expression

[W3-1] Coordination of Stress-Mediated Gene Expression and DNA Replication by Signaling Kinases

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Exposure of cells to osmostress results in the activation of the Hog1 stress-activated protein kinase (SAPK). Activation of this highly conserved MAP kinase is required to generate a set of osmoadaptive responses essential for cell survival. Adaptation to osmostress requires the induction of a large number of genes as well as the control of cell cycle progression. Upon stress, there is a major down-regulation of gene expression that is bypassed specifically in stress-responsive genes by the action of the Hog1 SAPK which acts in multiple steps of mRNA biogenesis including transcription initiation and elongation (Nadal-Ribelles et al., 2012; Nadal-Ribelles et al., 2014; Nadal-Ribelles et al., 2015). In addition to regulate transcription, SAPKs control cell cycle progression. During S phase, the Hog1 SAPK is critical to coordinate transcription and replication by directly phosphorylating Mrc1, allowing for full stress-responsive transcription without affecting DNA integrity (Duch et al., 2013). Remarkably, in addition to osmostress, other stresses also induce a large number of genes during S phase. These transcriptional outbursts cause genomic instability and transcription-associated recombination in cells carrying a non-phosphorylatable Mrc1 mutant. By a systematic biochemical assay we have identified several kinases that are able to phosphorylate Mrc1 in the same phosphorylation sites than Hog1. This indicates that Mrc1 can integrate signals from multiple kinases to delay replication when an outburst of transcription occurs during S phase. All together highlights the relevance of the signaling kinases in the control of gene expression and cell cycle regulation.

Keywords: *SAPK signaling, cellular stress, gene transcription and cell cycle regulation*

[W3-2] Epigenetic Control Of Aging In Response To Caloric Restriction

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Changes in histone post-translational modifications have been implicated in the aging process. These epigenetic alterations are an attractive model through which environmental signals, such as diet, could be integrated in the cell to regulate its lifespan. However, evidence linking dietary interventions with specific alterations in histone modifications that subsequently affect lifespan remains elusive. We show that deletion of the epigenetic

enzyme N-alpha-terminal acetyltransferase Nat4 and loss of its associated histone H4 N-terminal acetylation (N-acH4) extends yeast cellular lifespan. Notably, the longevity induced by deletion of Nat4 is epistatic to the effects of calorie-restriction (CR). Consistent with this, i) Nat4 expression is downregulated and the levels of N-acH4 within chromatin are reduced upon CR, ii) constitutive expression of Nat4 and maintenance of N-acH4 levels reduces the extension of lifespan mediated by CR, and iii) transcriptome analysis indicates that loss of Nat4 largely mimics the effects of CR, especially in the induction of stress-response genes. We further show that nicotinamidase Pnc1, which is typically upregulated under CR, is required for the longevity mediated by Nat4 inactivation. Collectively, these findings establish the epigenetic enzyme Nat4 and H4 N-terminal acetylation as novel regulators of cellular aging that link CR to increased stress resistance and longevity.

Keywords: *Replicative lifespan, Epigenetic modifications, Caloric restriction*

[W3-3] How Yap8 couples arsenic-sensing to transcriptional regulation of target genes

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The transcription factor Yap8 is critical for arsenic tolerance in the yeast Saccharomyces cerevisiae [1]. Yap8 is a member of the evolutionary conserved AP-1 family of bZIP transcription factors, and is the first arsenic sensor protein described in eukaryotes [2]. Currently, little is known about the molecular mechanisms by which Yap8 couples arsenic-sensing to transcriptional regulation of target genes. Our data are consistent with a model in which a DNA-bound form of Yap8 acts directly as an arsenite [As(III)] sensor, and that As(III) binding to Yap8 acts as a molecular switch that converts inactive Yap8 into an active transcriptional regulator [2]. Preliminary data indicates that Yap8 triggers chromatin remodelling to efficiently induce gene expression: nucleosome scanning, chromatin immunoprecipitation and gene expression assays show that histone occupancy decreases whilst RNA polymerase II occupancy and target gene expression increase in an As(III)- and Yap8-dependent manner. Taken together, our work has shed novel light on Yap8 function and advanced our molecular understanding of one of the largest families of transcription factors in eukaryotic cells.

Keywords: *arsenite, metalloid, arsenic sensing, Yap8, transcription factor, gene expression, AP-1 family, yeast*

[W3-4] Dal80 GATA factor intragenic occupancy is independent of its target sequences in the ORF and correlates with high mRNA abundance in budding yeast

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Yeast nitrogen catabolite repression (NCR) is a paradigm for metabolic adaptations to environmental changes. Key factors involved in this process are four GATA transcription factors (Gln3, Gat1, Dal80 and Gzf3), which are active in conditions of nitrogen limitation (proline), bind upstream of NCR-sensitive genes and regulate their expression. Although the consensus binding site for these factors has been characterized a while ago and is dispersed throughout the genome, the precise set of regulated genes was still lacking. We performed a genome-wide analysis of Dal80 binding (ChIP-Seq), correlated our results with GATA site occurrence and with GATA factor-regulated gene expression (RNA-Seq) in proline-grown cells. ChIP-Seq analyses revealed that Dal80 binds to ORFs in addition to gene promoters and this binding correlates with high gene expression. ORF binding was confirmed at a large set of characterized NCR-sensitive genes, and we showed that this occurred as a consequence of elongation, independently of GATA sequences that may be present in the ORF.

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Keywords: *Transcription, ChIP-Seq, NCR*

[W3-5] Specialized Yeast Ribosomes to Develop Methods for Therapeutic Readthrough of Premature Termination Codons in Rare Disease

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Ribosomes execute codon directed mRNA translation into proteins. Mutations that alter sense mRNA codons into unscheduled stop codons are termed premature termination codons (PTCs), also called non-sense codons. A PTC triggers the nonsense-mediated mRNA decay pathway and production of a truncated, potentially deleterious protein. PTC mutations account for about 25% of the known 8000 genetic diseases and most of those fall into the class of rare diseases, i.e. are identified in less than 1% of the population.

In severe cases of PTC alleles on muscular dystrophy and cystic fibrosis, respectively, administration of high doses of aminoglycosides has achieved PTC readthrough by reducing accuracy of decoding in the A-site of the ribosome, albeit with severe side effects to the patients (1). We show here that the versatile yeast translation system can be used to identify methods for therapeutic readthrough of premature termination codons.

Our approach demonstrates that altering the functional availability of individual ribosomal proteins (2) or that of individual rRNA nucleotide modifications (3) generates specialized ribosomes, which show preferential translation of selected mRNAs, including PTC reporters,

in a background of largely unaltered bulk translation. We performed a specialized yeast ribosome screen by employing a large collection of diploid yeast strains, each deficient in one or other copy of the set of ribosomal protein genes, generating distinct populations of altered “specialized” ribosomes. Comparative protein synthesis assays were used to assess translational readout of different, heterologous mRNA reporters, including PTC reporters.

We identified ribosomal protein rpL35/uL29 as specific target for therapeutic intervention and repair of the human LAMB3-PTC mRNA. PTC induced loss of Lamb3 protein causes severe blistering of the skin and rare skin disease JEB-H (Herlitz junctional epidermolysis bullosa), as the trimeric laminin 5 complex - linking epidermis and dermis - can’t be formed in the absence of Lamb3 protein. We have developed, optimized and validated a cellular keratinocyte assay to investigate the impact of small molecules binding to rpL35/ uL29 to form a specialized ribosome for LAMB3-PTC repair.

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2. Bauer J.W., et al., PLoS One. 2013.

3. Schosserer M., et al., Nat Commun. 2015.

Keywords: *Specialized Ribosomes, PTC readthrough, Rare disease*

[W3-6] Function and Factors of mRNA Stability Changes Upon a Nitrogen Upshift

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Changes in mRNA stability can hasten the reprogramming of the yeast transcriptome to adapt to sudden environmental changes. We measured the changes in mRNA degradation rates of the whole yeast transcriptome in response to the relief of nitrogen-limitation (glutamine pulse upshift) using 4-thiouracil pulse-chase labeling and RNA sequencing. We find that accelerated degradation contributes to the rapid repression of Nitrogen Catabolite Repression (NCR) transcripts. Surprisingly, the degradation rate of some mRNAs encoding enzymes involved in carbon metabolism are also accelerated in response to a nitrogen upshift. At the same time, we find evidence for stabilization of mRNAs encoding the enzymes of the GMP biosynthesis pathway and some components of ribosome biogenesis machinery. To identify regulators of accelerated mRNA degradation, we screened for factors that impair accelerated degradation of GAP1 mRNA - a highly abundant transcript that is subject to NCR control. To accomplish this we developed a novel method integrating mRNA FISH, FACS, and sequencing (FFS) of molecular barcodes in a highly-multiplexed pooled screen of the prototrophic yeast deletion collection. We identified factors involved in mRNA quality control that contribute to this accelerated degradation of GAP1 mRNA, suggesting a possible role for mRNA quality control in mediating post-transcriptional remodeling of the transcriptome in dynamic environments.

Keywords: *mRNA dynamics, mRNA degradation, mRNA regulation, high-throughput screen, nitrogen-upshift, NCR, GAP1*

[W4] Metabolism and organelles

[W4-1] Systematic Identification and Functional Characterization of Contact Site Resident Proteins in Saccharomyces cerevisiae

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Contact sites are areas of close apposition between organelle membranes that enable the transfer of lipids, metabolites and small molecules in an efficient manner. In recent years, several new membrane contact sites have been described and the tethering proteins that hold the two membranes together have been identified. Despite these advances we are still far from having a deep understanding of the function and regulation of most contact sites. To mechanistically characterize a contact site it is essential to know its entire repertoire of resident proteins yet very few proteins that are enriched in any contact site have, to date, been described. To systematically characterize the proteome of contact sites we have decided to utilize a panel of split fluorescence sensors for a diversity of contact sites in Saccharomyces cerevisiae. With this sensor one part of a fluorophore is fused to the outer membrane of one organelle while the second is fused to another organelle's membranes. If a contact site is present between both membranes, a fluorescent signal is emitted. We have taken sensors for 15 contacts between several organelles such as mitochondria, peroxisomes, lipid droplets, vacuoles, the plasma membrane and the endoplasmic reticulum, and crossed these split-tagged strains with a novel library of mCherry tagged yeast proteins. By analyzing co-localization events we have discovered a large number of new, previously unappreciated, contact site residents. Following up on these proteins, especially those that are conserved to humans, should give us a fresh look at the diversity of functions performed at contact sites and how this affects eukaryotic physiology.

Keywords: *Contact sites, high content screens, proteomics*

[W4-2] Glucose and calcium-responsive phosphatases mediate crosstalk between organelle and plasma membrane proton pumps

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In S. cerevisiae, coordinated activity of the intracellular V-ATPase proton pump responsible for organelle acidification and the plasma membrane proton pump Pma1 is critical for pH homeostasis. Both chronic loss of V-ATPase activity in vma mutants and acute loss through treatment with V-ATPase inhibitors result in ubiquitination of Pma1 by the Rsp5 E3-ubiquitin ligase acting in combination with α-arrestin, Rim8. Approximately 50% of Pma1 is then internalized and degraded in the vacuole. Failure to internalize Pma1 when V-ATPase activity is lost causes very poor growth, indicating that endocytic downregulation of Pma1 is compensatory (Smardon and Kane (2014) J. Biol. Chem. 289:32316). The mechanisms for

communicating loss of V-ATPase activity to plasma membrane Pma1 and designating 50% of Pma1 pumps for endocytosis are not understood. The negative genetic interaction between mutations required for Pma1 internalization and vma mutations allowed us to uncover additional candidates involved in this signaling pathway. Mutations in the Ca2+/calmodulin-dependent protein phosphatase calcineurin were previously shown to be synthetically lethal with vma mutants. We obtained vma2Δcnb1Δ mutants that grew very weakly at pH 5, and found that Pma1 is not internalized. Treatment of a vma2Δ mutant with calcineurin inhibitor FK506 resulted in inhibited growth, reduced Pma1 ubiquitination, and accumulation of Pma1 at the plasma membrane. The vma2Δ mutation was also synthetically lethal with the glc7-12ts mutation at semi-permissive temperatures and with a reg1Δ mutant, which lacks a regulatory subunit of the Glc7 (PP1) phosphatase. The vma2Δ glc7-12ts double mutant compromised Pma1 ubiquitination and retained Pma1 at the plasma membrane upon a shift to the non-permissive temperature. These data indicate that loss of V-ATPase activity is signaled to Pma1 through multiple pathways capable of sensing cytosolic Ca2+, glucose metabolism, and pH. We also asked whether simply reducing the level of cell surface Pma1 in a vma2Δ mutant would bypass the need for ubiquitination and endocytosis. However, a pma1-007 mutation, which reduces Pma1 levels by 50%, is synthetically lethal in combination with vma2Δ; this lethality was not suppressed by preventing Pma1 endocytosis with a rim8Δ mutation. This suggests that compensation for loss of V-ATPase activity may require populating endocytic compartments with Pma1, rather than simply reducing plasma membrane Pma1 activity.

Keywords: *proton transport, pH regulation, endocytosis, arrestin, calcineurin, vacuole, ubiquitination*

[W4-3] Metabolic Reprogramming and Stress Resistance Elicited by Glucose Starvation is Coordinated to Extend Chronological Lifespan in Yeast

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Calorie restriction is the only non-genetic means which can effectively extend lifespan and healthspan from yeast to mammals. Although a number of evolutionarily conserved signalling pathways, such as IGF-1 and TOR, are implicated in the regulation of lifespan mediated by calorie restriction, our understanding of the underlying mechanisms is still poor. Using stress response reporters whose expressions are induced by glucose limitation and starvation, we have screened the whole gene deletion library and identified many genes that are necessary to mount an effective defence against oxidative and heat stresses. They fall into major clusters of signalling pathways, mitochondrial function, r-RNA processing, DNA damage and repair, transcription from RNA polymerase and cell cycle regulation. Subsequent analyses of the ‘signalling’ mutants not only revealed novel regulators of chronological lifespan (CLS), such as the GSK-3 ortholog Mck1, but also demonstrated that starvation signals transmitted by SNF1/AMPK, PKC1 and those negatively

regulated by TOR/PKA (including PAS kinase Rim15, DYRK kinase Yak1 and Mck1) are integrated to enable metabolic reprogramming and the acquisition of stress resistance. The coordination of the metabolic shift to respiration ensures the conversion of toxic fermentation products (ethanol and acetate) to storage carbohydrates, which are used as energy stores for quiescent cells to maintain viability. Our findings suggest that the key to extend lifespan lies with the ability (and the opportunity) to metabolically adapt to different nutrient conditions (metabolic flexibility) and to overcome redox stress associated with metabolic reprogramming to maintain proteostasis.

Keywords: *glucose starvation, chronological lifespan, metabolic reprogramming, stress resistance*

[W4-4] A Regulated Protein Aggregation Controls Glucose Response in Yeast

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Utilization of non-fermentable carbon sources requires the activity of the Snf1 protein kinase. Snf1 is active in the absence of glucose and regulates the expression and activity of proteins involved in respiration. We have identified two new regulators of Snf1 activity in S. cerevisiae. These new regulators control the localization of the Snf1 activator Std1 in response to glucose into reversible aggregates, via the Std1's asparagine-rich region. Interestingly, reversible Std1 aggregation occurs under non-stressful, ambient conditions, creating inclusion bodies in the form of a liquid drop, and utilizes the Hsp40, Hsp70 and Hsp104 chaperones, similarly to the aggregation of toxic or misfolded proteins such as those associated with Parkinson’s, Alzheimer’s and CJD diseases. This suggests that protein aggregation mechanisms are a normal, non-pathological physiological state that can be used to regulate central metabolic processes. Our results reveal a controlled, non-pathological, physiological role of protein aggregation in the regulation of a major metabolic cellular pathway and have implications for our understanding of cancer and neurodegenerative diseases.

Keywords: *reversible protein aggregation, glucose metabolism, chaperones*

[W4-5] Lysine acetyltransferase NuA4 and acetyl-CoA regulate glucose-deprived stress granule formation in Saccharomyces cerevisiae

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Eukaryotic cells form stress granules under a variety of stresses, however the signaling pathways regulating their formation remain largely unknown. We have determined that the Saccharomyces cerevisiae lysine acetyltransferase complex NuA4 is required for stress granule formation upon glucose deprivation but not heat stress. Further, the Tip60 complex, the human homolog of the NuA4 complex, is required for stress granule formation

in cancer cell lines. Our work suggests that NuA4 is regulating glucose-deprived stress granule dynamics through two distinct pathways – regulating Acetyl-CoA levels and direct acetylation of stress granule proteins. Surprisingly, the impact of NuA4 on glucose-deprived stress granule formation is partially mediated through regulation of acetyl-CoA levels, which are elevated in NuA4 mutants. While elevated acetyl-CoA levels suppress the formation of glucose-deprived stress granules, decreased acetyl-CoA levels enhance stress granule formation upon glucose deprivation. We found that NuA4 modulates acetyl-CoA levels through the regulation of Acetyl-CoA Carboxylase Acc1. We have also determined that NuA4-dependent lysine acetylation of the core stress granule component poly-A-binding protein Pab1 at lysine 131 contributes to the formation of glucose-deprivation stress granule assembly. Altogether this work establishes that NuA4 regulates the formation of glucose-deprived stress granules through acetylation of Pab1 and through regulation of Acc1 and the metabolite acetyl-CoA.

Keywords: *Stress Granules, Lysine acetyltransferase, NuA4, Pab1, glucose deprivation, Acc1, acetyl-Coa*

[W4-6] Acyl-Coa synthetases activate Fatty Acids on forming Autophagosomes to drive Autophagy

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Macroautophagy is a key intracellular stress response, in which autophagosomes form de novo as double-membrane vesicles and transport enclosed cytoplasmic cargo to vacuoles for degradation. Core autophagy machinery drives poorly defined membrane rearrangements underlying autophagosome biogenesis. Here we identify dedicated long-chain acyl-CoA synthetases (ACS), conserved proteins that activate fatty acids (FA) by coenzyme A-linkage, as part of the autophagy machinery in Saccharomyces cerevisiae. Determined by N-terminally encoded targeting information, ACS localized to nucleated autophagic membranes and progressively accumulated on forming autophagosomes. Cells deficient for local FA activation were severely compromised in autophagy caused by a reduced rate and extended duration of autophagosome formation, and nonproductive autophagosome-vacuole fusion. Our work demonstrates that mechanisms of FA channelling directly drive key stages of autophagy, and provides insights into the fundamental processes of autophagosome biogenesis.

Keywords: *Autophagy, Lipid Metabolism, Proteostasis, Organelle dynamics, Ageing, Quality Control, Fatty acid*

[W4-7] An In Vitro TORC1 Kinase Assay that Recapitulates the Gtr-independent Glutamine-responsive TORC1 Activation Mechanism on Yeast Vacuoles

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Evolutionarily-conserved TOR complex 1 (TORC1) is an essential regulator of cell growth, which responds to nutrients, especially amino acids. TORC1 is activated by amino acids through multiple types of sensing machinery with different specificities for each amino acid in yeast and mammals. In Saccharomyces cerevisiae, leucine, and perhaps other amino acids, was reported to activate TORC1 via the heterodimeric small GTPases Gtr1-Gtr2, the orthologs of the mammalian Rag GTPases. More recently, an alternative Gtr-independent TORC1 activation mechanism that may respond to glutamine was reported, although its molecular detail is not clear.

In studying the nutrient-responsive TORC1 activation mechanism, the lack of an in vitro assay hinders associating particular nutrient compounds with the TORC1 activation status, whereas no in vitro assay that shows nutrient responsiveness has been reported. In this study, we have developed a new in vitro TORC1 kinase assay that reproduces, for the first time, the nutrient-responsive TORC1 activation. This in vitro TORC1 assay recapitulates the previously predicted Gtr-independent glutamine-responsive TORC1 activation mechanism. Using this system, we found that this mechanism specifically responds to L-glutamine, resides on the vacuolar membranes, and involves a previously uncharacterized Vps34-Vps15 phosphatidylinositol 3-kinase complex and the PI(3)P-binding FYVE domain-containing vacuolar protein Pib2. Pib2 interacted with TORC1 in response to L-glutamine on the isolated vacuolar membrane, suggesting that Pib2 is directory involved in the glutamine-responsive mechanism. Thus, the in vitro assay we have developed is proved to be useful for dissecting the glutamine-responsive TORC1 activation mechanism.

Keywords: *TORC1, rapamycin, amino acid, vacuole*

[W4-8] Ypq2, the Yeast Ortholog of Human Lysosomal PQLC2 Cationic Amino Acid Exporter, is a high-affinity transporter catalyzing passive arginine transport across the vacuolar membrane

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Ypq1, -2, -3 are highly similar transporters of the PQ-loop family known to be localized at the yeast vacuolar membrane (1). Several previous observations – e.g. transcriptional repression of YPQ3 under lysine excess conditions (1), high turnover of Ypq1 upon lysine starvation (2), resistance of ypq2 mutant to canavanine (a toxic analog of arginine) (1), reduced ATP-dependent uptake of basic amino acids in vacuolar membrane vesicles isolated from ypq mutant (3) - suggest a role of Ypq proteins in transport of cationic amino acids (arginine, lysine, histidine) across the vacuolar membrane. PQLC2 is a mammalian ortholog of the yeast Ypq proteins. Biochemical characterization of PQLC2 expressed to the cell surface of oocytes showed that the protein is a lysosomal exporter of basic amino acids. Furthermore, PQLC2 was found to play a central role in therapy against cystinosis, a lysosomal storage disease, by mediating export from the lysosome of a drug derivate resembling lysine (1). As the yeast vacuole is known to accumulate high amounts of basic

amino acids including arginine, we sought to further investigate the role of Ypq proteins in arginine transport across the vacuolar membrane. We have implemented the previously described methods used for isolating intact vacuoles (still containing their sap) and we set up conditions for measuring the activity of passive and active amino acid transporters. We will present data showing that Ypq2 is a high-affinity transporter catalyzing passive transport of arginine and histidine across the vacuolar membrane. Work is in progress to assess the role of Ypq2 in vacuolar export of arginine under normal and starvation conditions. As PQLC2 expressed in yeast is able to complement the phenotype of an ypq2 mutant (1), we hope that our study of Ypq2 will shed more light on the physiological role of PQLC2 in human cells.

Keywords: *vacuole, cationic amino acid transporter, Ypq2, PQLC2*

[W5] New tools in yeast research

[W5-1] High Resolution QTL Mapping of Ethanol Tolerance in Saccharomyces cerevisiae Using Advanced Intercross Lines (AIL) and Selective DNA Pooling (SDP)

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Ethanol, the end product of fermentation in Saccharomyces cerevisiae, is the main biofuel used worldwide. Since ethanol is toxic to the yeast cell, ethanol production is inhibited by its accumulation. Therefore, understanding the genetic basis of ethanol tolerance is important for the development of improved yeast strains with higher ethanol tolerance and yields. However, up to now, genomic elements affecting ethanol tolerance have only been mapped at low resolution, hindering their identification.

Here, we used Advanced Intercrossed Line (AIL) design, to perform high resolution mapping of QTLs affecting ethanol tolerance in yeast.

Selective DNA Pooling (SDP) and whole-genome sequencing were used in F6 of a cross between two widely separated S. cerevisiae haploid strains. Fifty and 95 QTLs affecting growth and survival, respectively, were identified by applying uniquely developed statistical methods.

The median size of a QTL Region (QTLR) was 12.1 Kb (growth) and 10.5 Kb (survival). These QTLRs are much narrower than previous reports. Importantly, some QTLRs included only a single gene. The mapping revealed significantly enriched biological processes important for ethanol tolerance in S. cerevisiae.

Keywords: *Ethanol tolerance, QTL mapping, Saccharomyces cerevisiae*

[W5-2] High-Quality Yeast Genome Assembly And Efficient Structural Variant Detection Using Nanopore Sequencing

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Comprehensive genomic variant maps are essential to explore genome evolution as well as its phenotypic consequences in natural populations. To date, short-read sequencing allowed to have genome- and species-wide views of mainly single nucleotide and copy number variants as we recently obtained in the Saccharomyces cerevisiae species by whole genome

sequencing of 1,011 natural (http://1002genomes.u-strasbg.fr/) isolates using an Illumina technology. However, the detection of structural variants (e.g. long indels, inversions, translocations) (SVs) still poses challenges, more precisely when variants are in high complexity regions while they correspond to genetic variants underlying phenotypic variation. Emerging long-read sequencing technologies, such as Oxford Nanopore MinION sequencing, provide an unprecedented opportunity to efficiently detect these structural variants. To evaluate the performance of this technology for whole-genome assembly and SVs detection, we resequenced various genomes of natural isolates of two distinct yeast species, namely Saccharomyces cerevisiae and Dekkera bruxellensis, showing different degree of genomic complexity. Using the ONT MinION at moderate coverage (~20x), highly complete and contiguous assemblies have been obtained. Data generated allowed hence to accurately detect SVs, such as translocations and large inversions throughout the genomes. Among the long inserted and deleted regions, we identified those related to transposable elements and could provide a complete cartography of these elements among the sequenced isolates. Our results clearly show the value of the MinION system for screening whole genomes for complex SVs and deeply characterizing genome architecture in yeast natural populations.

Keywords: *structural variant detection, Oxford Nanopore MinION sequencing, whole-genome assembly*

[W5-3] How To Observe Single Molecules Of Transcription Factors In Yeast Saccharomyces

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In vivo Single Molecule Tracking (SMT) has recently developed into a powerful technique for measuring and understanding the transient interactions of transcription factors (TF) with their chromatin response elements. The role that this rapid exchange plays in transcription remains hotly debated. Is this noise, or is it transcriptionally productive? This method was successfully applied to mammalian cells but in yeast it remains problematic. Key technical problems are (a) a lack of robust procedures to determine if the labeling strategies used to mark the transcription factor are innocuous and therefore to ensure that SMT estimates are correct; (b) low retention of the bright and stable organic dyes covalently binding to HaloTag used in the state-of-the art SMT experiments; (c) the small size of the yeast nucleus, which is ~10-fold smaller in diameter than mammalian cells, and therefore the imaged area is ~100-fold smaller. To facilitate the dye incorporation we disrupted ATP-binding cassette multidrug resistance (ABC-MDR) transporter PDR5. We developed new and robust procedure for evaluation of adverse effects of labeling, and new quantitative analysis procedures that significantly improve residence time measurements by accounting for fluorophore blinking. We estimated residence time of the yeast transcription factor Ace1p on non-specific and specific chromatin sites. Estimates of TF binding to specific sites clarify the function of the fast exchange and provide a deeper understanding of the molecular mechanisms of transcription initiation. Our results provide a framework for the reliable performance and analysis of SMT of transcription factors in yeast.

Keywords: *Single Molecule tracking, HaloTag, Fluorescence microscopy, Saccharomyces, Transcription Factors, CUP1, ACE1*

[W5-4] Application of FAP Technology to Study α-Arrestin-mediated Receptor Endocytosis in S. cerevisiae

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G protein-coupled receptors (GPCRs) are activated by many different types of extracellular stimuli, especially various kinds of chemical agonists. Binding of such a ligand to its cognate GPCR activates signal transduction pathways and thereby causes appropriate responses. In yeast, the mating pheromone α-factor binds to and activates signaling emanating from the GPCR Ste2. However, hyperstimulation of Ste2 can cause cell death. Therefore, cells have evolved mechanisms that act at the receptor level to prevent excess signaling. Among the feedback controls responsible for down-regulation of cellular response to GPCR-initiated signaling is removal of the receptor from the plasma membrane (PM) via clathrin-mediated endocytosis. Studies in yeast by others first showed that this internalization process involves marking α-factor-bound Ste2 for internalization by ubiquitinylation mediated by the PM-associated HECT domain-containing ubiquitin ligase Rsp5. However, recent work in our laboratory has shown that α-factor-induced ubiquitinylation of agonist-activated Ste2 by Rsp5 requires either of two intermediary "matchmaker" proteins, the α-arrestins Rod1 and Rog3. PPxY motifs in the C-terminal segments of these endocytic adaptor proteins bind Rsp5 and their N-terminal arrestin fold domains engage Ste2. To provide an assay to dissect the recognition determinants in both the receptor and these α-arrestins required for their productive interaction, we have been applying an innovative new method, called fluorogen-activating protein (FAP) technology, to visualize Ste2 localization. A fluorogen is an organic molecule that is relatively non-fluorescent when free in solution; however, when the fluorogen is bound to a cognate FAP [a human single-chain antibody (scFvs) that has been engineered to bind the fluorogen with high affinity and specificity], the fluorogen-FAP complex is highly fluorescent. To "catch α-arrestins in action", we co-express a GFP-tagged Rog3 allele, Rog3(Δ400-733) (called Rog3Δ400, for short), which lacks its PPxY motif-containing C-terminal tail (and also lacks phosphorylation sites that we think may help them dissociate from their targets) in cells expressing FAP-tagged Ste2 that have been treated with fluorogen. Cells are then exposed to α-factor to examine whether the fluorescent α-arrestin and fluorogen-FAP-Ste2 co-localize at the PM and other aspects of the dynamics of agonist-induced Ste2 endocytosis. Results from such studies will be presented.

Keywords: *G protein-coupled receptor, α-arrestin, endocytosis*

[W5-5] The Protein Architecture of the Yeast Endocytic Machinery Analyzed by FRET

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Clathrin-mediated endocytosis is a principal vesicle trafficking route from the plasma membrane to endosomal compartments. It is essential for membrane homeostasis, recycling of numerous membrane proteins and signaling. To form an endocytic vesicle from a small piece of the plasma membrane dozens of proteins assemble at the endocytic site in a highly coordinated manner. Although the timeline of their assembly is known, their functional arrangement at the endocytic site is still poorly understood. Such information however is critical to mechanistically understand the process of endocytic vesicle formation and its regulation.

Analysis of the protein architecture of the endocytic site is a complicated task given that multiple copies of up to 50 proteins localize in this diffraction-limited spot for a limited time. Importantly, protein densities at the endocytic site are very well suited for mapping its organization by Förster/fluorescence resonance energy transfer (FRET). Here we show that FRET, a powerful tool to detect proximities between fluorescently labeled proteins separated by less then 10 nm, can be successfully used to analyze the dynamic architecture of the endocytic site.

To map the functional organization of the endocytic machinery we performed a systematic FRET-based proximity screen of 17 conserved endocytic proteins. We obtained multiple highly specific protein proximity pairs, many of them not yet recognized by other (e.g. protein interaction) methods. Further mapping using the clathrin subunits and truncated protein variants as spatial rulers allowed us to draw a detailed map of the yeast endocytic site. Selected protein proximity pairs have been then used to investigate the protein rearrangements occurring during endocytic vesicle invagination by real-time FRET imaging methods. Altogether, our studies show FRET as a highly valuable tool to study protein architectures of molecular machines with the yeast endocytic machinery as an example.

Keywords: *vesicle trafficking, membrane trafficking, endocytosis, live cell imaging, FRET*

[W5-6] Combining the Mother Enrichment Program with High-Throughput Replica-Pinning to Identify Genes that Suppress the Accumulation of Mutations

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Defects in genes whose products act to maintain DNA integrity lead to genome instability, resulting in an overall increase of the spontaneous mutation rate. This condition, known as “mutator phenotype”, is thought to be a driving force during tumorigenesis.

An S. cerevisiae cell divides asymmetrically, yielding an (increasingly) ageing mother cell and a fully rejuvenated daughter cell. The Mother Enrichment Program (MEP) is an estradiol-inducible genetic system which allows daughter cell-specific inactivation of two

essential genes, thus enabling the analysis of a cohort of ageing mother cells. When the MEP is active, daughter cells irreversibly arrest at the G2/M transition, while the mother cells keep dividing, resulting in a linear growth rate and the formation of microcolonies on agar medium. Occasionally, some cells become capable of evading the MEP due to acquired mutations: even in the presence of estradiol, these cells, called escapers, grow exponentially and form normal colonies on plates.

We developed a high-throughput replica-pinning approach to estimate spontaneous mutation rates in yeast strains, where estradiol-insensitive escaper colony formation serves as a readout for the occurrence of spontaneous mutations. With this protocol, we performed a genome-wide screen for genome maintenance genes. The MEP system was introduced in the yeast deletion collection using Synthetic Genetic Array technology. High-density arrays of MEP colonies were then grown for one week on agar plates in the presence of estradiol to allow for accumulation of spontaneous mutations in mother cells throughout their lifespan. Subsequently, colonies were further replicated on estradiol plates to detect the occurrence of escapers. High-throughput analysis of escaper formation frequency allowed identification of deletion mutants with elevated spontaneous mutation rate. Among many known mutator genes, we also found new genes involved in the maintenance of genome integrity.

Keywords: *Genome stability, Mutagenesis, Mother enrichment program, High-throughput screen*

[W5-7] The Daughter Extinction Program (DEP): a synthetic biology approach to explore the genetic and metabolic landscape of mitotic ageing

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Eukaryotic cells can only undergo a limited number of cells divisions, which defines the mitotic age of the cell. Although extensively investigated, high-throughput assays to assess mitotic ageing are still lacking. To address this limitation, we developed the Daughter Extinction Program (DEP) in the yeast Saccharomyces cerevisiae, which inducibly arrest cell cycle in daughter cells. Using two independent, daughter cell-specific, inducible systems, we were able to selectively prohibit cell division in daughter cells of prototrophic, diploid yeast cells. As our DEP renders an exponential growing population to a linear function of dividing mother cells, measurements of cell densities allow us to determine median mitotic lifespan, thereby clearing the way for fully automated, high-throughput measurements. Using synthetic genetic array (SGA)-technology, we introduced our DEP into the existing yeast libraries (YKO and Damp) and for the moment, we are evaluating mitotic ageing. Moreover, our system is perfectly equipped to evaluate the effect of different metabolic conditions as well as chemical compounds on mitotic ageing, allowing for n-dimensional functional clustering of each gene and straightforward identification of compounds affecting mitotic ageing.

Keywords: *mitotic ageing, system-wide approach, new yeast research tool*

[W5-8] Phenotypic Profiling in Yeast Using High-content Screening and Automated Image Analysis

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We have developed experimental and computational pipelines which combine array-based yeast genetics and automated microscopy for systematic and quantitative cell biological screens or phenomics. In one project, we use the Synthetic Genetic Array (SGA) method to introduce fluorescent markers of key cellular compartments or cell cycle progression, along with sensitizing mutations, into yeast mutant collections. We then perform live cell imaging on the mutant arrays using HTP confocal microscopy to quantitatively assess the abundance and localization of our fluorescent reporters, providing cell biological readouts of specific pathways and cellular structures in response to thousands of genetic perturbations. For automated image analysis, we developed a hybrid computational pipeline that combines outlier detection and classical SVM-driven phenotype labeling, as well as a neural network-based approach. Our neural network, DeepLoc, was able to classify highly divergent image sets, highlighting deep learning as an important tool for expedited analysis of high-content microscopy data.

Keywords: *functional genomics, image analysis, cell biology*

[W6] Cell cycle, cytoskeleton and morphogenesis

[W6-1] The adder phenomenon emerges from independent control of pre- and post-Start phases of the budding yeast cell cycle

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While it has long been clear that cells actively regulate their size, the molecular mechanisms underlying this regulation have remained poorly understood. In budding yeast, cell size primarily modulates the duration of the cell division cycle by determining the timing of the G1/S transition known as the Start. We have recently shown that cell growth dilutes the cell cycle inhibitor Whi5 in G1 to increase the rate of progression through Start. However, recent phenomenological studies in yeast and bacteria have shown that cells increase approximately a fixed volume during their entire cell cycle, independent of what size they are born. These results seem to be in conflict, as the phenomenological studies suggest that cells measure the amount they grow, rather than their size, and that size control acts over the whole cell cycle, rather than specifically in G1. Here, we propose an integrated model that unifies the adder phenomenology with the molecular mechanism of G1/S cell size control. We use single cell microscopy to parameterize a full cell cycle model based on independent control of pre- and post-Start cell cycle phases. We find that our model predicts the size-independent volume increase during the full cycle. This suggests that the adder phenomenon is an emerging property of several aspects of the budding yeast cell cycle rather than a causal consequence of an underlying molecular mechanism measuring a fixed volume increase.

Keywords: *size control, cell cycle, cell growth*

[W6-2] Extreme Calorie Restriction in Yeast Retentostats Induces Uniform Non-Quiescent Growth Arrest

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Non-dividing Saccharomyces cerevisiae cultures are highly relevant for fundamental and applied studies. However, cultivation conditions in which non-dividing cells retain substantial metabolic activity are lacking. Unlike stationary-phase (SP) batch cultures, the current experimental paradigm for non-dividing yeast cultures, cultivation under extreme calorie restriction (ECR) in retentostat enables non-dividing yeast cells to retain substantial metabolic activity and to prevent rapid cellular deterioration. The use of these tightly controlled yeast retentostats has allowed detailed characterization of the transition from slow to virtually no growth at the level of (quantitative) physiology, transcriptome and proteome.

These prior studies have, however, studied retentostat cultures at the whole culture level, while cells in SP-cultures are known to differentiate into different cell-types. The aim of the current studies was therefore to investigate heterogeneity in yeast cultures transitioning towards cell division arrest under ECR.

Cellular DNA content analyses and visualization of actin structures showed that the majority of cells resided in G1 of the cell cycle. In sharp contrast to starved cultures, including SP cultures, only few cells (< 10%) contained F-actin bodies in continuous cultures, independent of the average culture doubling time. Furthermore, no subpopulations with distinct cellular HSP12 or HSP26 transcript concentrations, genes known to be differentially expressed in different cell types in stationary phase cultures, could be detected under ECR based on smRNA FISH analyses.

These findings show that retentostat cultivation yields highly homogeneous and robust cultures, in which cells most likely survive in an extended G1 phase. Furthermore, it demonstrates that yeast cells exposed to ECR differ from carbon-starved cells and offer a promising experimental model for studying non-dividing, metabolically active, and robust eukaryotic cells.

Keywords: *Saccharomyces cerevisiae, Cell-division arrest, extreme calorie restriction, Heterogeneity, mRNA FISH, Actin structure, Retentostat*

[W6-3] DNA Circles Cause Nuclear Pore Complex Rearrangements during Yeast Aging

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Saccharomyces cerevisiae confines aging factors in the mother cells during mitosis, in order to ensure the emergence of a rejuvenated and naïve daughter cell. The daughter cell obtains a full replicative potential, while the aging factors in the mother cell cause cellular dysfunction and increased mortality rate. One prominent aging factor in budding yeast is the presence of non-centromeric DNA circles (1). They are formed by homologous recombination during DNA repair. Different studies show that DNA circles interact via SAGA with nuclear pore complexes (NPCs), and that these NPCs cluster together to stay in the mother cell during mitosis (2, 3). However, how these circles contribute to aging is unknown. We wondered whether DNA circle binding through SAGA cause alterations in NPCs and if this could affect cell viability.

We observed that NPCs having a DNA circle anchored lose the basket structure, which normally protrudes into the nucleoplasm. Basket detachment, and specifically loss of Nup60, is required to anchor DNA circles to the NPC in order to confine them to the mother cell. Basket fixation to the pore or targeting to the DNA circle prevents their interaction with the NPC and releases them from retention in the mother cell. Mutations in SAGA show a same loss of circle retention and are long-lived. We conclude that DNA circles require basketless NPCs to be asymmetrically retained in the aging mother cell. In agreement, we observed a progressive accumulation of basketless NPCs during aging.

These basketless and aged pores fail to recruit the SUMO protease Ulp1. Fixing Ulp1 to the NPC or targeting it to the DNA circle fully destabilizes their interaction with NPCs and their retention in the mother cell, even in the absence of Nup60. The basketless pores fail to recruit Ulp1 via Nup60, thereby preventing desumoytion of targets involved in DNA-NPC interaction. We suggest that its exclusion from NPCs is needed for a stable interaction between DNA circle and NPC, assuring that circles remain confined to the mother cell.

Altogether, DNA circles seem to cause an alteration of the NPC structure during aging, which is needed for their retention. Furthermore, the accumulation of modified NPCs might be a major cause of increased mortality with age.

1) Sinclair DA, Guarente L. Cell. 1997

2) Denoth-Lippuner A, Barral Y, et al. Elife. 2014

3) ShcheprovaZ, Barral Y, et al. Nature. 2008

Keywords: *aging, Nuclear pore complex, mitosis, DNA, post-translational modification*

[W6-4] Actin Filament Initiation and Regulation at Cell Membranes

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Actin nucleation is the key rate limiting step in the process of actin polymerization, and tight regulation of this process is critical to ensure actin filaments form only at specific regions of the cell. Arp2/3 is a well-characterised protein complex that can promote nucleation of new filaments, though its activity requires additional nucleation promotion factors. The best recognized of these factors are the WASP family of proteins that contain binding motifs for both monomeric actin and for Arp2/3. Previously we demonstrated that the yeast WASP homologue, Las17, in addition to activating Arp2/3 can also nucleate actin filaments de novo, independently of Arp2/3. This activity is dependent on its polyproline rich region. Through biochemical analysis we have identified key motifs within the polyproline region that are required for nucleation and for elongation of actin filaments in the absence of Arp2/3. When expressed in cells, Las17 harbouring mutations in these motifs, causes changes in the behaviour of endocytic reporter proteins prior to Arp2/3 recruitment, supporting a role early in actin filament initiation. Other experiments have allowed us to identify key phosphorylation sites involved in regulating actin binding, and regions of the protein conferring lipid binding properties. Overall our data support a model in which Las17 interacts with primed endocytic sites to directly generate and tether new actin filaments.

Keywords: *cytoskeleton, actin, actin-nucleation, WASP family proteins, membranes*

[W6-5] The Pathway of Pulling Yeast Nuclei in Anaphase Controls Nuclear Motility at All Cell Cycle Stages in a Multinucleated Yeast

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Today, biologists want to understand the mechanisms of subsystems in cells. Biochemistry, for a long time extremely successful in developing amazing in vitro systems, can no longer handle mechanistic questions above a certain complexity. The only way to move forward is to use all experimental data gathered in the past to reconstruct the system in a mathematical model.

Our work demonstrates this approach by studying the coordination of movements of multiple nuclei in a filamentous yeast which evolved from budding yeast. Two pulling mechanism operate in S. cerevisiae, the Kar9-Bim1-Myo2 pathway for positioning of nuclei at the bud neck prior to nuclear division and the Dynein-Num1 pathway during pulling of nuclei through the bud neck. In the obligatory filamentous yeast Ashbya gossypii nuclei are highly motile performing bi-directional short- and long-range movements including nuclear by-passing. Homologs for all S. cerevisiae components involved in nuclear positioning and pulling are expressed in A. gossypii. However, experimental data accumulated over the past years had only identified the dynein motor, increased microtubule growth and shrinkage rates, budding yeast-like spindle pole bodies as microtubule organization centers, and Num1 (cortical dynein anchor) as key elements for the observed complex nuclear movements in A. gossypii.

This knowledge was implemented to run for the first time realistic simulations of movements of multiple nuclei in a common cytoplasm, which generated in vivo-like nuclear migration patterns. The simulations could also verify mutant phenotypes. We then exploited the simulation set up to study the influence of the number of microtubules per nucleus, an increase in cytoplasmic flow, and changes in organelle concentration on nuclear motility.

Keywords: *Evolution of nuclear dynamics, Microtubule dynamics, Dynein, Simulations*

[W6-6] Casting light on the genome of the cell's powerhouse: The distribution and inheritance of mitochondrial DNA

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Essential subunits of the mitochondrial respiratory chain, which generates the majority of energy in eukaryotic cells, are encoded in the mitochondrial genome (mtDNA) that is present in hundreds of copies in every cell. Mutations within mtDNA have been identified as the cause for a multitude of human diseases and have been tightly linked to the ageing process and altered stem cell homeostasis. Accordingly, to ensure organismal health, good copies of mtDNA have to be faithfully inherited during cell division, their integrity needs to be maintained over generations and they need to be distributed throughout the mitochondrial network to provide all mitochondrial segments with mtDNA encoded

proteins. Astonishingly, it remains poorly understood how cells accomplish these fundamental tasks.

By exploiting the unique advantage that mtDNA can be manipulated in S. cerevisiae, we developed a novel system that for the first time allowed minimally invasive tracking of mtDNA in living cells. Using this system, we have gained unique insights into the cellular principles that govern distribution and inheritance of mtDNA and the maintenance of its integrity. We found that nucleoids are non-randomly spaced within the mitochondrial network and observed the spatio-temporal events involved in mtDNA inheritance. Surprisingly and against the prevailing view, cells deficient in mitochondrial fusion and fission distributed and inherited mtDNA normally, pointing to alternative pathways involved in these processes. We identified such a mechanism, where we observed fission-independent, but F-actin–dependent, mitochondrial tip generation that was linked to the positioning of mtDNA to the newly generated tip. Although mitochondrial fusion and fission were dispensable for mtDNA distribution and inheritance, we showed through a combination of genetics and next-generation sequencing that their absence leads to an accumulation of mitochondrial genomes harboring deleterious structural variations. These data support a role for mitochondrial fusion and fission in maintaining the integrity of the mitochondrial genome by facilitating cleansing of mutant mtDNA copies.

Keywords: *Mitochondria, mitochondrial DNA, mtDNA, S. cerevisiae, Mitochondrial dynamics, Live cell microscopy*

[W6-7] Role of Cdc42 Pathways in Regulating Group Cooperation and the Transition to Differentiated Multicellularity

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Group cooperation provides an evolutionary benefit and is thought to underlie evolutionary processes including the transition to multicellular life. The decision-making process surrounding group cooperation is poorly understood. Here, we identify decision-making pathways as key regulators of group cooperation. By exploring filamentous growth in unicellular fungal microorganisms, we describe a new response where cells assemble into multicellular aggregates to promote nutrient foraging. The Rho GTPase Cdc42, its effector MAPK pathway, and other signaling pathways regulated aggregate formation. Aggregates assembled by a mechanism where filaments from different groups interlocked to knit groups together, which resembled the intercalation of cells into tissues during mammalian development. This system was used to model the impacts of cheaters and handicapped individuals in aggregate assembly. Probing the limits of group cooperation showed that individuals adopt an optimal range of social interactions to balance foraging needs. The uncanny resemblance between Cdc42 pathways in regulating group social responses in yeast and tissue development in metazoans led to the idea that decision-making pathways, by promoting group cooperation, may have contributed to the transition to multicellular life. This theory was supported by the roles GTPase pathways played in differentiated multicellular phenotypes in a laboratory selection experiment.

Keywords: *Rho GTPases, polarity, fungal pathogenesis, multicellular development, filamentous growth, signal transduction, Cdc42, MAPK*

[W7] Proteostasis, ageing and disease models

[W7-1] Physiological Regulation of Heritable Protein Aggregation

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Ordered protein aggregates (amyloids) and their transmissible variety (prions) are associated with important human diseases. In yeast, self-perpetuating protein isoforms, termed yeast prions, control heritable traits. Prion formation and loss are modulated by environmental and physiological conditions. Our data show that a heat-induced metastable prion, formed by an actin assembly protein, may persist in a fraction of yeast cells for a significant number of cell generations after return to normal growth conditions, thus carrying a cellular memory of stress. Moreover, evolutionary acquisition of a prion-forming ability by this protein coincides with the acquisition of increased thermotolerance in the Saccharomyces lineage (Chernova et al., 2017 Cell Reports 18: 751). Propagation of yeast prions is controlled by the same cytosolic chaperones that are responsible for the protection of yeast cells against a proteotoxic stress. Chaperones fragment prion polymers and generate new oligomeric seeds, promoting new rounds of aggregation. Therefore, yeast prions are adjusted to physiological levels of chaperone proteins and hijack a cellular stress defense machinery for their own propagation. Chaperones of the ribosome associated complex, that are involved in proper folding of a nascent polypeptide, antagonize initial prion formation. During a stress, the decrease in overall translational activity is accompanied by a relocation of the ribosome associated chaperones into a cytosol, resulting in an impairment of a prion-like propagation of misfolded proteins. Cellular apparatus, controlling the asymmetry of a mitotic division, influences maintenance and properties of self-perpetuating protein aggregates both during recovery from stress and in the process of replicative aging. Overall, intimate relationship with the protein quality control machinery of the cell plays a key role in the processes of prion formation and propagation in yeast. (Supported by grants MCB 1516872 from NSF, and 14-50-00069 from RSF.)

Keywords: *Amyloid, Chaperone, Prion, Protein Quality Control, Stress*

[W7-2] Mechanisms of Prion-Dependent Lethality In Yeast

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Prions are self-perpetuating protein conformations which often have an amyloid nature. In yeast amyloid prions manifest as heritable traits as their presence affects normal function of prion proteins. E.g., [PSI+], the prion form of translation termination factor Sup35 (eRF3), decreases accuracy of stop codon recognition and exhibits nonsense-suppressor phenotype. Additional production of Sup35 in [PSI+], but not [psi-] cells, is toxic. This phenomenon is referred to as prion-dependent lethality or prion toxicity.

We screened for Q/N-rich transcription factors affecting [PSI+] propagation and found that excess of several factors enhanced [PSI+]-dependent lethality. The lethality was accompanied by elevation in SUP35 mRNA levels in [PSI+] strain, suggesting transcriptional upregulation as the mechanism. One of the factors, Sfp1, was shown not only to upregulate SUP35, but also SUP45, while additional expression of SUP45 is known to alleviate the [PSI+] toxicity. We showed that, indeed, SUP45 overexpression compensated [PSI+]-dependent lethality caused by excess Sup35, but did not affect Sfp1-derived lethality, implying involvement of another mechanism. Search for the factors that influenced the lethality of excess Sfp1 led to identification of Hsp40 chaperone Sis1 which alleviated toxicity of excess of both Sfp1 and Sup35 in [PSI+] strains. Sfp1 colocalized with [PSI+] aggregates and its overproduction also affected Sup35 aggregate size, while coexpression of Sis1 returned the size distribution to normal. Thus at least two different mechanisms are involved in [PSI+]-dependent lethality.

Sis1 is known to counter the toxicity of many prions and amyloids in yeast, such as Rnq1/[PIN+] and [PIN+]-dependent polyQ-toxicity, but the underlying mechanisms are not entirely understood. Recently we showed that partitioning of Sis1 between cellular compartments may affect different prions in different ways. [PSI+] is less sensitive to alterations is Sis1 than other prions. Relocalization of most cellular Sis1 into the nucleus causes [PSI+]-dependent growth defect, accompanied by enhancement of [PSI+], suggesting that cytoplasmic Sis1 normally aids in detoxifying [PSI+] aggregates, allowing non-lethal [PSI+] propagation.

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Keywords: *yeast prions, prion toxicity, [PSI+], regulation of transcription, Sfp1, Sis1*

[W7-3] Metals and Metalloids Cause Protein Misfolding and Aggregation

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Certain metals and metalloids, such as cadmium and arsenite, are common environmental pollutants. They are considered highly toxic and are classified as human carcinogens. While their toxicity is undisputed, the underlying in vivo molecular mechanisms are not fully understood. Here, we demonstrate that cadmium, like arsenite, induces aggregation of cytosolic proteins in living yeast cells. Cadmium primarily targets proteins in the process of synthesis or folding, probably by interacting with exposed thiol groups in not yet folded proteins. Cells that cannot efficiently protect the proteome from cadmium-induced aggregation or clear the cytosol from protein aggregates are sensitized to cadmium. Thus, protein aggregation may contribute to cadmium toxicity.

Using a high-content imaging screen, we identified processes that control protein aggregation upon As(III) exposure. We demonstrate that blocking transcription during As(III) exposure prevents protein aggregation. Furthermore, we show that a loss in transcriptional control leads to enhanced protein aggregation and As(III) toxicity. Interestingly, the molecular chaperone Sse1p acts as buffer in cells that cannot properly regulate transcription already in the absence of As(III), but even more so during As(III) exposure. Altogether, our findings suggest that the loss of transcriptional control leads to accumulation of protein aggregates, thereby contributing to As(III) toxicity.

Keywords: *Metal toxicity, Protein aggregation, Protein folding*

[W7-4] Prion-like Properties of a Yeast G Protein Receptor Involved in Regulation of Mating

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G-protein-coupled receptors (GPCRs) are integral membrane proteins that initiate responses to extracellular stimuli by mediating ligand-dependent activation of cognate heterotrimeric G proteins. Ste18 is a gamma-subunit of a G-protein receptor that is conserved in evolution and plays a key role in a variety of cellular processes, including pheromone-signaling pathway that is crucial for the yeast mating. We demonstrate that Ste18 possess prion-like properties. Upon overproduction, Ste18 forms detergent-resistant amyloid-like aggregates and promotes formation of [PSI+], a prion isoform of another protein, Sup35/eRF3. Ste18 mutants, defective in anchoring to plasma membrane, are not able to form detergent-resistant aggregates or induce [PSI+] prion, while a mutant, deficient in signal transduction but not in membrane anchoring, is able to do so. These data show that prion-like properties of Ste18 depend on its association with a membrane and resemble our previous results for another protein, Lsb2 (see Chernova et al., 2017 Cell Reports 18: 751-761), whose prion properties depend on association with a peripheral actin cytoskeleton. Overall, our findings emphasize the significance of a specific intracellular location for prion formation. Similar to Lsb2, Ste18 is ubiquitinated, short-lived and degraded by a proteasome. Levels of Ste18 protein are increased in the conditions when proteasome function is impaired, suggesting

that aggregate formation by Ste18 may occur in response to the malfunctioning or overload of the ubiquitin-proteasome system, for example during a proteotoxic stress. Potential involvement of prion-like aggregation in regulation of G-protein dependent signaling and yeast mating will be discussed in the light of both our data and recent developments, suggesting the role of protein aggregation not only in diseases, but also in regulation of some biological processes.

Keywords: *G-protein, prion, amyloid, proteasome, aggregation, ubiquitin*

[W7-5] Proteostasis Impairment and Endoplasmic Reticulum Stress in a Yeast Model for Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis is a neurodegenerative disease that affects motor neurons. The majority of ALS cases (90%) are sporadic. More than thirty genes have been associated with familial cases so far. The P56S mutation in the protein VAPB was associated with ALS8 in Brazilian patients. VAPB is a membrane protein of the endoplasmic reticulum that is possibly involved in diverse cellular functions, including intracellular trafficking, interaction endoplasmic reticulum-Golgi and Unfolded Protein Response. Here, we aimed to analyze the endoplasmic reticulum stress and protein degradation pathways as factors underlying the pathogenicity of VAPB. The BY4741 strain of the yeast Saccharomyces cerevisiae expressing VAPBWT or VAPBP56S was employed as a model. Initially, it was verified that both VAPBWT and VAPBP56S were located in the endoplasmic reticulum, and the VAPBP56S protein was found as SDS-resistant aggregates. Expression of the VAPBP56S but not of VAPBWT was toxic, reducing cell viability. Since proteasome and autophagy are two processes that protect cell against toxicity associated with aggregates, the effects of their inhibition were investigated. Proteasome was inhibited by the use of the MG 132 compound (under the Δpdr5 background), reducing the viability of strains expressing VAPBWT and the rate of VAPBWT degradation. In contrast, autophagy inhibition (achieved by atg8 gene deletion) affected mainly the viability of cells expressing VAPBP56S. Cells expressing VAPBWT and VAPBP56S displayed increased levels of proteasome subunits. As expected, in cells expressing VAPBWT the proteasome activity was increased and the pool of ubiquitilated proteins was diminished. Surprisingly, in cells expressing VAPBP56S proteasome activity was reduced and ubiquitilated proteins accumulated at much higher levels. The autophagosomes formation (assessed by GFP-Atg8 fusion) was increased only in strains expressing VAPBP56S, suggesting higher levels of autophagy. Finally, the levels of endoplasmic reticulum stress markers (pdi1, ero1, lhs1 and kar2) were induced in cells expressing VAPBP56S. Taken together, our data suggest a link between proteostasis, endoplasmic reticulum stress and VAPBP56S in our ALS model, with autophagy playing a predominant role in the protection against toxicity associated with aggregates formation.

Keywords: *Amyotrophic Lateral Sclerosis, Proteostasis, Endoplasmic Reticulum Stress, Disease Model, Proteasome, Autophagy*

[W7-6] Budding yeast HECT_2 protein Ipa1 is an essential ubiquitin-protein ligase influencing proteasome activity

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The ubiquitin-proteasome system (UPS) controls cellular functions by maintenance of a functional proteome and degradation of key regulatory proteins. Central to the UPS are ubiquitin-protein ligases that selectively catalyze modification of target proteins with ubiquitin, thereby controlling their abundance or initiating regulatory events. Here, we demonstrate that the essential Saccharomyces cerevisiae protein Yjr141w/Ipa1 (Important for cleavage and PolyAdenylation) is a HECT_2 (homologous to E6-AP carboxyl terminus_2) -type ubiquitin-protein ligase. We identified residues within the HECT_2 family signature that are essential for Ipa1 function and provide evidence for the importance of the C-terminal region, which has low similarity to HECT-type ubiquitin-protein ligases. In agreement with a function as ubiquitin-protein ligase, Ipa1 shows interactions with several ubiquitin-conjugating enzymes in vivo and exhibits autoubiquitylating activity in vitro. The activity of the nuclear localized Ipa1 is important for cell cycle progression and cell size control. Loss of Ipa1 function reduces proteasomal degradation of several substrates and results in activation the Rpn4 regulon. Our results demonstrate that Ipa1 is an ubiquitin-protein ligase, which influences proteasome activity in yeast.

Keywords: *Ubiquitin proteasome system, Protein degradation, Ubiquitin-protein ligase*

[W7-7] Amino Acid Substitution Equivalent to Human Chorea-acanthocytosis I2771R in Yeast Vps13 Protein Affects its Binding to Phosphatidylinositol 3-phosphate

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The rare human disorder chorea-acanthocytosis (ChAc) is caused by mutations in hVPS13A gene. The hVps13A protein interacts with actin and regulates the level of phosphatidylinositol 4-phosphate (PI4P) in membranes of neuronal cells. Yeast Vps13 is involved in vacuolar protein transport and, like hVps13A, participates in PI4P metabolism. Vps13 proteins are conserved in eukaryotes, but their molecular function remains unknown. One of the mutations found in ChAc patients causes amino acids substitution I2771R which affects the localization of hVps13A in skeletal muscles. To dissect the mechanism of pathogenesis of I2771R, we created and analyzed a yeast strain carrying the

equivalent mutation. Here we show that in yeast, substitution I2749R causes dysfunction of Vps13 protein in endocytosis and vacuolar transport, although the level of the protein is not affected, suggesting loss of function. We also show that Vps13, like hVps13A, influences actin cytoskeleton organization and binds actin in immunoprecipitation experiments. Vps13-I2749R binds actin, but does not function in the actin cytoskeleton organization. Moreover, we show that Vps13 binds phospholipids, especially phosphatidylinositol 3-phosphate (PI3P), via its SHR_BD and APT1 domains. Substitution I2749R attenuates this ability. Finally, the localization of Vps13-GFP is altered when cellular levels of PI3P are decreased indicating its trafficking within the endosomal membrane system. These results suggest that PI3P regulates the functioning of Vps13, both in protein trafficking and actin cytoskeleton organization. Attenuation of PI3P-binding ability in the mutant hVps13A protein may be one of the reasons for its mislocalization and disrupted function in cells of patients suffering from ChAc.

Keywords: *Vps13, chorea-acanthocytosis, yeast model, actin cytoskeleton, binding of lipids, protein trafficking*

[W7-8] Uncovering Las17 secret service in nucleolus

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The yeast orthologue of the Wiskott-Aldrich Syndrome protein, Las17, is a key regulator of cortical actin polymerization. Disruption of LAS17 leads to the loss of actin patches and to block in endocytosis. Unexpectedly, when we used ASPIC-MS methodology to identify proteins bound to yeast chromosome XII containing rDNA repeats, aberrantly migrating on PFGE as chromosome cloud, we detected Las17. Using Las17-GFP fusion protein and the nucleolar marker Nop1-DsRed we confirmed the nucleolar localization of Las17. Further experiments comparing las17Δ mutant and the wild type strains revealed differences in nucleolar integrity, shape and positioning during cell division, suggesting a role of Las17 in nucleolar organization and division. The las17Δ cells showed also higher than wild type cells nucleolar fragmentation score after nocodazole treatment. Because Las17 overexpression protects cells against various stresses that affect the nucleolus, such as starvation, cell wall stress or oxidative stress, we postulate that the involvement of Las17 in cell growth regulation, extrachromosomal DNA transportation and stress responses is linked to its role in nucleolar organization. We believe that newly assigned role of Las17 as a nucleolar protein engaged in nucleolar function, most likely nucleolar division, and ensuring nucleolar integrity might help to elucidate the etiology of the human immunodeficiency Wiskott-Aldrich Syndrome.

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Keywords: *Las17, Saccharomyces cerevisiae, Wiskott-Aldrich Syndrome, nucleolus, aberrant DNA structure, DNA-binding protein*

[W8] Stress signalling and protein trafficking

[W8-1] The Yeast Mep2 Ammonium Transceptor Physically Interacts With The 14-3-3 Protein Bmh1

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

Keywords: *Pseudohyphal growth, ammonium sensing, transceptor, stress signaling*

[W8-2] H+-Influx Coupled to Amino Acid Uptake as a Key Signal Stimulating TORC1 in Saccharomyces cerevisiae

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The Target Of Rapamycin Complex 1, TORC1, is a kinase complex conserved from yeast to mammals that controls cell growth by fine-tuning anabolic and catabolic processes. Among the various signals that impinge on TORC1 are amino acids, which modulate TORC1 activity through the conserved Rag family GTPases. In mammalian cells, the intracellular concentration of specific amino acids is sensed by different sensor systems modulating the Guanine nucleotide Exchange Factors (GEF) and GTPase Activating Proteins (GAP) acting on the Rag GTPases. Yet in yeast the upstream regulators of the Rag GTPases (named Gtr1 and Gtr2) responding to amino acids remain poorly known. In the context of our study of the

role of TORC1 in the mechanisms promoting the ubiquitylation and endocytosis of the yeast general amino acid transporter, Gap1, we have found that the uptake of ß-alanine by Gap1 activates TORC1 in a manner dependent on the Rag/Gtr GTPases. Yet ß-alanine cannot be used as a nitrogen source and HPLC measurements showed that it is not converted into other amino acids. On the basis of these observations, we explored different hypotheses to account for TORC1 activation in response to ß-alanine uptake. For instance, the Gap1 transporter could act as a transceptor capable of signaling to TORC1, or the process of ß-alanine transport by itself could somehow activate TORC1. We will present data indicating that it is the influx of protons associated with H+-coupled transport of ß-alanine that stimulates TORC1. We propose that this signal contributes to TORC1 reactivation accompanying exit of cells from various nutritional starvation conditions.

Keywords: *TORC1, Nutrient transporters, Protein trafficking*

[W8-3] Roles of SPS Pathway Genes (SSY1-PTR3-SSY1) and SIR3 During Cycles of Growth and Starvation in S. cerevisiae Populations With Differing Proportions of Quiescent and Non-Quiescent Cells

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Over its evolutionary history, Saccharomyces cerevisiae has evolved to be well-adapted to fluctuating nutrient availability. In the presence of sufficient nutrients, yeast cells continue to proliferate, but upon starvation haploid yeast cells enter stationary phase and differentiate into non-quiescent (NQ) and quiescent (Q) cells. Q cells survive stress better than NQ cells and show greater viability when nutrient-rich conditions are restored. To investigate the genes that may be involved in the differentiation of Q and NQ cells we serially propagated yeast populations that were enriched for either only Q or only NQ cell types over many repeated growth-starvation cycles. After 30 cycles (equivalent to 300 generations), each enriched population produced a higher proportion of the enriched cell type compared to the starting population, suggestive of adaptive change. We also observed differences in each population's fitness suggesting possible tradeoffs: clones from NQ-lines were better adapted to logarithmic growth, while clones from Q lines were better adapted to starvation. Whole genome sequencing of clones from Q and NQ enriched lines revealed mutations in genes involved in stress response and survival in limiting nutrients (ECM21, RSP5, MSN1, SIR4, IRA2) in both Q and NQ lines, but also differences between the two lines: NQ line clones had recurrent independent mutations affecting the SPS amino acid sensing pathway, while Q line clones had recurrent, independent mutations in SIR3 and FAS1. Our results suggest that both sets of enriched cell-type lines responded to common, as well as distinct selective pressures.

Keywords: *quiescence, experimental evolution, fluctuating environment*

[W8-4] Phosphorylation of Cth2 protein and its Grr1-dependent degradation are essential to maintain Cth2 proper levels required for optimal growth during the iron starvation

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Iron is an essential micronutrient for all eukaryotic organisms because it participates as a redox cofactor in a wide range of metabolic pathways. Despite its abundance, iron bioavailability for living organisms is highly restricted due to its low solubility at physiological pH. In response to iron limitation eukaryotic cells optimize iron utilization by repressing non-essential iron-consuming pathways and activating indispensable iron-dependent processes. In yeast Saccharomyces cerevisiae Cth2 protein, characterized by the presence of two Cx8Cx5Cx3Hx18Cx8Cx5Cx3H tandem zinc fingers (TZFs), binds to AU-rich elements (AREs) of many mRNAs encoding for iron-containing proteins, promoting their degradation and leading to a decrease in respiration and an increase in dNTP synthesis. Previous studies have demonstrated that yeast cells need to fine-tune the expression levels of Cth2 protein because its excess can be detrimental for growth. We show that Cth2 is a highly unstable protein phosphorylated at serine residues 65, 68 and 70 under iron-deficient conditions. Mutagenesis of these serine residues does not eliminate Cth2 targeted mRNA degradation function, but it increases Cth2 protein stability. The F-box E3-ligase protein Grr1 is crucial to tightly regulate Cth2 protein levels because Grr1 recognizes phosphorylated Cth2 protein and facilitates it degradation by the proteasome. Both mutagenesis of Cth2 serine residues and GRR1 deletion give rise to yeast cells with significant growth defects under iron deficient conditions, emphasizing the physiological relevance of regulating Cth2 protein abundance.

Keywords: *Cth2, Iron deficiency, Grr1, Phosphorylation, Degradation*

[W8-5] Genetic Adaptive Mechanisms Mediating Response and Tolerance to Acetic Acid Stress in the Human Pathogen Candida glabrata: Role of the CgHaa1-dependent Signaling Pathway

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The increased resilience of Candida glabrata to azoles and the continuous emergence of strains resistant to other antifungals demands the development of new therapeutic approaches focused on non-conventional biological targets. Genes contributing to increase C. glabrata competitiveness in the different infection sites are an interesting and unexplored cohort of therapeutic targets. To thrive in the vaginal tract and avoid exclusion C. glabrata cells have evolved dedicated responses rendering them capable of tolerating multiple environmental challenges, including the presence of acetic and lactic acids produced by the commensal microbiota. In this work it is shown that the CgHaa1

transcription factor (ORF CAGL0L09339g) controls an acetic acid-responsive system essential for survival of C. glabrata in presence of acetic acid at a low pH. mRNA profiling showed that the genes up-regulated by CgHaa1 under acetic acid stress are involved in multiple physiological functions including membrane transport, metabolism of carbohydrates and amino acids, regulation of the activity of the plasma membrane H+-ATPase and adhesion. Consistently, under acetic acid stress CgHaa1 increased the activity and the expression of the CgPma1 proton pump and enhanced colonization of vaginal epithelial cells by C. glabrata. The protective effect of CgHaa1 against acetic acid was also linked to the reduction of the accumulation of the acid inside C. glabrata cells, this being partly dependent on the up-regulation of the multidrug transporter CgTpo3. Comparison of the CgHaa1-dependent regulatory network active in C. glabrata with the corresponding Saccharomyces cerevisiae orthologue network revealed prominent differences, consistent with the idea that the two pathways have evolved divergently with the CgHaa1 pathway suffering a “functional expansion”. The role of the CgHaa1-pathway in the extreme acetic acid-tolerance exhibited by vaginal C. glabrata isolates will also be discussed.

Keywords: *stress response and signalling in C. glabrata, Transcriptional regulation, Evolution of regulatory networks, Acetic acid stress tolerance*

[W8-6] Unraveling the Regulation of the Yeast Ppz1 Phosphatase by the Moonlighting Protein Hal3

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The Ser/Thr protein phosphatase (PPase) Ppz1 is an enzyme related to the ubiquitous type-1 PPases (PP1c) but it is found only in fungi (including pathogenic ones). Ppz1 controls monovalent cation metabolism and it is regulated by an inhibitory subunit, Hal3, which binds to the C-terminal catalytic domain of the phosphatase. Overexpression of Ppz1 is highly toxic for yeast cells, so its de-regulation has been proposed as a target for novel antifungal therapies.

While modulation of PP1c by its many regulatory subunits has been extensively characterized, the manner by which Hal3 controls Ppz1 remains unknown.As a first approach, we have constructed by PCR-prone mutagenesis a library of Ppz1 variants and we have developed a functional assay in search of mutations affecting the binding or/and the inhibitory capacity of Hal3. We have characterized in vivo and in vitro diverse Ppz1 versions carrying single or double amino acid mutations and found that, although in most cases they were clearly refractory to Hal3 inhibition, none of them exhibited significant loss in binding to the inhibitor. Mapping of mutations strengthened the notion that Hal3 does not interact with Ppz1 through its RVxF-like motif (found in most PP1c regulators). In contrast, construction of a 3D model of the C-terminal domain of Ppz1 based on existing related structures revealed that functionally relevant mutations mapped in a conserved acidic and hydrophobic substrate binding channel used by mammalian Inhibitor 2- to regulate PP1c. However, Hal3 does not bind nor inhibit yeast PP1c (Glc7). Therefore, these results indicates that modulation of PP1c and Ppz1 by their regulatory subunits likely

differs, although could share some structural features. The differential characteristics would allow insulation of Glc7 and Ppz1 functions in the yeast cell.

Keywords: *protein phosphatases, regulatory subunits, monovalent cation homeostasis, mutagenesis analysis, functional screen*

[W8-7] The Hsp70 SSB is a new actor in endocytic patch dynamics

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While molecular chaperones are primarily known for their role in protein folding, they have also been shown in recent years to participate in a large variety of cellular functions. Here, we establish for the first time that the Hsp70 SSB plays a novel role in the endocytic process. Endocytosis depends on an extensive network of interacting proteins that has been characterized in detail, but the mechanisms that regulate the spatio-temporal dynamics of the endocytic pathway is not fully understood. We demonstrate here that in the absence of SSB, cells exhibit a severe defect in endocytosis, the endocytic coat proteins assemble in aberrant structures, and the dynamics of the endocytic particles is slower. Our results show that SSB is necessary for the structure and function of several components of the endocytic pathway, in a step necessary for their progression to the late internalization stages. These results define a novel role for Hsp70s, distinct from the classical role in clathrin uncoating. Our study also clearly demonstrates that chaperones have key roles to play in cellular processes beyond basic protein folding, coordinating the assembly and disassembly of large protein complexes such as the endocytic particles.

Keywords: *molecular chaperones, Hsp70, endocytosis, yeast*

[W8-8] Characterizing Vacuolar Protein Sorting Pathways in the Yeast Pichia pastoris (Komagataella spp)

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Besides being frequently used as host for production of recombinant secretory proteins, the yeast Pichia pastoris (syn. Komagataella spp) is also used as a model system to study the secretory path¬way. This is due to the observation that in contrast to the commonly used yeast model Saccharomyces cerevi¬siae, where the Golgi is distributed throughout the entire cell, P. pastoris forms ordered Golgi stacks similar to the mammalian Golgi, which are located next to discrete transitional ER sites.

The further steps of vesicular transport in P. pastoris, in particular the protein sorting pathways towards the vacuole, were not well characterized so far. Thus, we set out to identify genes involved in these vesicular transport steps and to generate mutants in the vacuolar protein sorting pathways. In these strains, we investigated the changes in secretion of native vacuolar and recombinant secretory fluorescent reporter proteins as well as their intracellular localization. Vacuolar morphology was assessed by fluorescence microscopy. Also in P. pastoris, vacuolar targeting occurs through the well-described CPY and ALP pathways which are named after one of their transported cargo proteins (carboxypeptidase Y and alkaline phosphatase, respectively). Disruption of either of the two Golgi-to-vacuole pathways can be complemented by the other pathway. Interestingly, several genes that are non-essential in S. cerevisiae proved to be essential in P. pastoris, indicating differences between the two yeasts. Furthermore, synthetic lethality was observed for ALP and CPY components, as well as for genes involved in endosome-to-vacuole fusion and late steps of secretory transport. Furthermore, the impact of the vps mutant strains on secretion of heterologous proteins was investigated. Approaches how to efficiently adapt the host cell's secretion capacity will be presented, which confirm that impairment of vacuolar protein sorting is an effective means of enhancing secretion of heterologous proteins.

Taken together, these studies allowed us to gain comprehensive insights into the function and regulation of vacuolar and endosomal protein sorting pathways in P. pastoris. Apart from contributing to our understanding of intracellular transport, these strains proved be valuable tools for production of recombinant secretory proteins.

Keywords: *Pichia pastoris, protein secretion, vacuolar protein sorting, vacuolar transport*

[W9] Systems biology and bioinformatics

[W9-1] Quantitative metabolomics of a xylose utilising Saccharomyces cerevisiae strain expressing the Bacteroides thetaiotaomicron xylose isomerase on glucose and xylose

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The yeast Saccharomyces cerevisiae cannot utilize xylose, but the introduction of a few xylose isomerases function well in yeast, overcoming the limitations of the fungal oxido-reductive pathway. In this study, a diploid S. cerevisiae S288c[2n YMX1] strain was constructed expressing the Bacteroides thetaiotaomicron xylA (XI) and the Scheffersomyces stipitis xyl3 (XK) and the changes in the metabolite pools monitored over time. Cultivation on xylose generally resulted in gradual changes in metabolite pool size over time, whereas more dramatic fluctuations were observed with cultivation on glucose due to the diauxic growth pattern. The low G6P and F1,6P levels observed with cultivation on xylose, resulted in the incomplete activation of the Crabtree effect whereas the high PEP levels is indicative of carbon starvation. The high UDP-D-glucose levels with cultivation on xylose indicated that the carbon was channeled towards biomass production. The adenylate and guanylate energy charges were tightly regulated by the cultures, whilst the catabolic- and anabolic-reduction charges fluctuated between metabolic states. This study helped elucidating the metabolite distribution that takes place under Crabtree-positive and Crabtree-negative conditions when cultivating S. cerevisiae on glucose and xylose, respectively.

Keywords: *Metabolomics, Xylose isomerase, Bioethanol, S. cerevisiae*

[W9-2] Identifying Gene Targets for Improving L-Phenylacetylcarbinol Production in Saccharomyces Cerevisiae by In-Silico Aided Metabolic Engineering and In-Vivo Validation

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The use of microbes in industrial processes requires modification of natural metabolism in order to enhance efficiency and economic yield. As the cellular metabolism is a complex system, finding the best target genes whose manipulations can improve the biosynthesis of a desired product is not straightforward. Hence in metabolic engineering, whole metabolic network should be analyzed for identifying genes that affect the product formation. L-Phenylacetylcarbinol (L-PAC) is a precursor of several decongestant and antiasthmatic medicines such as ephedrine and pseudoephedrine. In yeast cells, pyruvate decarboxylase (PDC) is able to catalyze the condensation of exogenously added benzaldehyde and

endogenously produced pyruvate into L-PAC. Alcohol dehydrogenase (ADH) or other oxidoreductases are responsible for the accumulation of benzyl alcohol and 1-phenyl-1,2-propanediol (PAC-diol) as unwanted by-products. In this work, a genome-scale metabolic model and flux balance analysis were used for identifying new target genes to improvement this biotransformation. The effect of gene deletions on the flux distributions in the metabolic model of S. cerevisiae was assessed using OptGene and minimization of metabolic adjustments (MOMA). Six single gene deletion strains, Δrpe1, Δpda1, Δadh3, Δadh1, Δzwf1 and Δpdc1, were predicted in-silico and further tested in-vivo by using knock-out strains cultivated semi-anaerobically on glucose and benzaldehyde as substrates. For all strains, except Δadh1, higher amounts of L-PAC were produced as compared to the corresponding wild-type cells. Δzwf1 stands for the highest L-PAC formation (2.48 g/l) by 2 g/l of benzaldehyde which is 88 % of the theoretical yield. Findings of the present study can provide guidance for future works on the strain improvement to increase PAC production and demonstrate the successful utilization of computationally guided genetic manipulation to increase metabolic capacity.

Keywords: *Flux balance analysis, Genome-scale modeling, Metabolic engineering, L-Phenylacetylcarbinol, Saccharomyces cerevisiae*

[W9-3] Allelic Variants of the GTR1 Gene, Involved in the TORC1 Signaling Pathway, Affect Nitrogen Transporters Gene Expression and Nitrogen Consumption in Saccharomyces cerevisiae During Alcoholic Fermentation

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The role of TORC1 signaling pathway is to detect nutritional signals, mainly nitrogen, and to coordinate cell growth. Among its targets are genes related to nitrogen transport and metabolism. Therefore, the adaptation of Saccharomyces cerevisiae to changes in the availability of nitrogen imply variations in the activity of this signaling pathway, which are important in the regulation of the expression of its targets genes. Consequently, the study of the TORC1 signaling pathway becomes relevant in certain areas such as wine fermentation, where one of its main problems is the deficiency of nitrogen sources, such as ammonium and amino acids, which leads to sugglish or stuck fermentation. Previous studies have highlighted the GTR1 gene as a candidate to explain differences in nitrogen consumption. The GTR1 gene encodes a GTPase participanting in the EGO complex responsable for stimulating TORC1 in response to the absence of amino acids. Therefore, different alleles of this gene could differentially affect the expression of target genes of TORC1, such as nitrogen transporters and consequently, impact on the nitrogen consumption. In this context, the objective of this work was evaluate the effect of allelic variants of GTR1 on the differential expression of nitrogen transporters and the nitrogen consumption in S. cerevisiae strains during alcoholic fermentation. The GTR1 gene was selected from QTL mapping performed to a tetraparental population, called SGRP-4X, whose parents belong to four distinct clusters: Wine/European, West African, North America and Sake. These allelic

variants were validated by reciprocal hemizygosity assay, which were fermented in synthetic must and determined the nitrogen content by HPLC. Expression of MEP1, MEP2, MEP3, GAP1, AGP1, DIP5, GNP1 and TAT2 by qPCR at three points of the alcoholic fermentation was evaluated. Differences were observed in the consumption of ammonium, arginine, glutamic, serine, threonine, alanine and glutamine when comparing the Wine and West African alleles. In general, the strain with West African allele showed lower expression of the genes evaluate during the first hours of the fermentation process, which could explain the differences in the consumption of the nitrogen sources. In conclusión, the West African allele of GTR1 affects the expression of the nitrogen transporters, possibly by a constant activation of TORC1.

Keywords: *TORC1 pathway, Allelic diversity, Saccharomyces cerevisiae, Alcoholic fermentation, Nitrogen consumption*

[W9-4] More Than One Way In - Three Gln3 Sequences Required To Relieve Negative Ure2 Regulation and Support Nuclear Gln3 Import

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Gln3 is responsible for Nitrogen Catabolite Repression-sensitive transcriptional activation in yeast. In nitrogen-replete medium, Gln3 is cytoplasmic and NCR-sensitive transcription repressed. In nitrogen-limiting medium, in cells treated with TorC1 inhibitor, rapamycin or glutamine synthetase inhibitor, methionine sulfoximine (Msx), Gln3 becomes highly nuclear and NCR-sensitive transcription derepressed. Previously, nuclear Gln3 localization was concluded to be mediated by a single nuclear localization sequence, NLS1. Here we show that nuclear Gln3-Myc localization is significantly more complex than previously appreciated. We identify three Gln3 sequences, other than NLS1 that are highly required for nuclear Gln3-Myc localization. Two of the sequences exhibit characteristics of mono- and bi-partite NLS sequences. Mutations altering these sequences are partially epistatic to a ure2 deletion. The third sequence, the Ure2 relief sequence, contains no NLS homology. Phosphomimetic aspartate substitutions for serine residues in the Ure2 relief sequence pleiotropically abolish nuclear Gln3-Myc localization in response to both limiting nitrogen and rapamycin treatment. In contrast, these Gln3 responses are normal in parallel serine to alanine substitution mutants. A ure2 deletion is epistatic to the aspartate to serine substitutions in the Ure2 relief sequence. These observations demonstrate that Gln3 responses to specific nitrogen environments potentially occur in multiple steps that can be genetically separated. At least one general step, that which is associated with the Ure2 relief sequence, may be prerequisite for responses to the specific stimuli of growth in poor nitrogen sources and rapamycin inhibition of TorC1. Supported by NIH grant GM-35642-27.

Keywords: *nitrogen catabolite repression, TorC1, Gln3, Ure2, rapamycin, methionine sulfoximine, nuclear import, protein phosphorylation*

[W9-5] Systems Level Study of S. cerevisiae Mutant Cells Demonstrating Different RNA Polymerase III Activity

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S. cerevisiae, efficiently fermenting sugar and producing ethanol, serves as a model for studying cancer. Both yeast and tumor cells rely on increased glucose uptake and high glycolytic activity. In addition, rapidly proliferating cancer cells not only accelerate glycolysis but also show abnormally elevated abundance of RNA polymerases I (RNAP I) and III (RNAP III) transcripts.

Regulation of RNAP III, which is responsible for tRNA synthesis in yeast S. cerevisiae in response to carbon source is mediated by general RNAP III- repressor, Maf1. Maf1 driven repression occurs on non-fermentable carbon source. The maf1 Δ strain has perturbations in gluconeogenesis [1]. The phenotypic effect of Maf1 deletion is suppressed by G1007A point mutation in the second largest RNAP III subunit C128 [1]

The two mutated strains have different preferences towards carbon sources.

The maf1 Δ, in which transcription of the gluconeogenic genes FBP1 and PCK1 is down regulated on glycerol, has reduced HXT6/7 transcript levels [3]. rpc128-1007 mutant, which grows poorly in the presence of a high concentration of glucose but has unperturbed glucose sensing via the major glucose signalling pathway Snf3/Rgt2, shows increased transcripts of HXT6/7 and HXT2 transporters, (which are considered to respond only to low glucose concentrations), regardless of the growth conditions either in the presence of fermentable or non-fermentable carbon source [2]

To survey proteome changes in relation to glucose utilization, we performed a comparative label-free quantitative study in response to high glucose concentration in maf1 Δ and rpc128-1007 strain. The changes in proteome demonstrate that the economy of protein production and glucose metabolism is central to cell physiology. Although glycolysis is accelerated in maf1 Δ it does not lead to an enhancement of ethanol production, channelling carbon to other metabolic end products.

The presented study provides an elegant example of using systems level approach in combination with targeted metabolic and molecular biology analysis, such as RT-PCR quantification of mRNA steady-state levels, for successful deciphering of metabolism in Saccharomyces that can further serve as a template for design of strains for increased production of industry-relevant commodities in yeast cells designed “a la carte”. This study was supported by National Science Centre, Poland grant no. 2012/05/E/NZ2/00583

[1] Ciesla MCB 2007, 27(21)7693

[2] Adamczyk PlosOne 2017

Keywords: *RNAP III activity, tRNA, glucose signaling, RNAP II gene regulation, metabolism, proteomics*

[W9-6] Comparative Analysis of Protein Abundance Studies to Quantify the Saccharomyces cerevisiae Proteome

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Global gene expression and proteomic tools have allowed large-scale analyses of the transcriptome and proteome in eukaryotic cells. These tools have enabled studies of protein abundance changes that occur in cells under stress conditions, providing insight into the regulatory programs required for cellular adaptation. While the proteome of yeast has been subjected to the most comprehensive analysis of any eukaryote, each of the existing datasets are separate and reported in different units. A comparison of all available datasets is key towards a complete understanding of the yeast proteome. We evaluated 19 quantitative proteomic analyses performed under normal and stress conditions and normalized and converted all measurements of protein abundance into absolute molecules per cell. Our analysis yields an accurate estimate of the cellular abundance of 92% of the proteins in the yeast proteome. We evaluate the variance and sensitivity associated with different measurement methods and explore the correlation of protein abundance with RNA sequencing and ribosomal profiling data. We find that C-terminal tagging of proteins has little effect on protein abundance. Finally, our normalization of diverse datasets facilitates comparisons of protein abundance remodeling of the proteome during cellular stresses.

Keywords: *Protein Abundance, Proteome, High-Throughput, Green Fluorescent Protein, Mass Spectrometry, Tandem Affinity Tag*

[W9-7] Integration of Structure- and Network-based Approaches for Network Pharmacology Prediction

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Drugs or drug candidates may interact with numerous molecules in the human body. Unexpected binding between compounds and proteins (i.e. off-target interactions) may result in unfavourable effects, leading therapeutic risks, negatively impacting drug development. The application of network pharmacology to predict molecular binding potential, drug reactions and toxicity caused by multiple targeting interactions is therefore expected as a promising method for evaluating polypharmacological effects. Here, we present systemsDock, a web server which integrates the results from molecular simulation and network-based analysis to assess binding potentials of a given small molecule (test compound) against proteins involved in a complex molecular signalling pathway. We

developed a predictive re-scoring function to assess molecular binding poses generated by docking tools and to rank them accordingly. In a validation of classifying test compound activity by using Receiver Operating Characteristic (ROC), we obtained the values of 75% sensitivity (i.e. compounds correctly identified as active) and 76% specificity (compounds correctly identified as inactive), showing a good performance on prediction of molecular binding potential.

We used the developed systemsDock system to screen numerous test compounds over Influenza A Virus Life Cycle pathway map (FluMap) for discovering anti-influenza agents. Together with the application of our re-scoring function, the proposed screening approach is able to comprehensively characterize the underlying mechanism of a drug candidate with good accuracy, improving the prediction of drug efficacy and safety. systemsDock is freely accessible at http://systemsdock.unit.oist.jp/.

Keywords: *Computer-aided drug design., Network pharmacology., Molecular simulation.*

[W9-8] Memory of stress response in S. cerevisiae

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When a cell undergoes an external stress, it responds by activating specific genes with a certain dynamics. In the budding yeast for instance, better tolerance or faster transcriptional response have been described for successive oxidative or nutritive stresses, respectively. Various hypotheses have been proposed to explain the mechanisms behind those different effects such as the influence of the chromatin or the involvement of specific cytoplasmic factors. But it still remains unclear how such memories are established.

To address those questions, we have investigated the response to hyperosmotic stresses in S. cerevisiae. We have used a microfluidic system to subjugate the cells to short repeated stresses while making single-cell measurement of the STL1 gene, tagged with fluorescence. This gene is located in the subtelomere of the chromosome IV, a domain of low transcriptional activity in non-stress conditions. We defined “mothers” the cells that receive the first stress and “daughters” the cells born from such stressed mothers. Our results show that the mothers adapt to repeated stresses and we have named this phenomenon “memory effect”. This memory effect is transmitted to the daughters, even though they have never experienced a stress before.

We have moved the gene of interest to a domain of higher transcriptional activity, which has interestingly led to a decrease in the gene’s activity upon stress and the loss of the memory effect. This suggests an involvement of the chromatin environment on the response to repeated stresses. Moreover, we have found a dynamical variability among cells which may or may not respond to several consecutive stresses. As shown by stress response modelling, this phenomenon could be explained by a variable delay among the yeast between the sensing of the stress and the start of the genetic response.

We found that even in response to short hyperosmotic stresses, the same population of yeast develop a memory of the stress, which is transmitted to the first generation of progeny. Our study show that this memory is gene-position dependent. This suggests an evolutionary organization of the stress response genes in the nucleus that might be involved in the emergence of phenomenon such as the memory effect. This work could serve as a basis to explain similar phenomena in other organisms.

Keywords: *memory, evolution, single-cell, microfluidics, variability, microscopy, nuclear organization*

Poster Sessions

[PS1] Autophagy and dynamics of organelles

[PS1-1] Casting light on the genome of the cell's powerhouse: The distribution and inheritance of mitochondrial DNA

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Essential subunits of the mitochondrial respiratory chain, which generates the majority of energy in eukaryotic cells, are encoded in the mitochondrial genome (mtDNA) that is present in hundreds of copies in every cell. Mutations within mtDNA have been identified as the cause for a multitude of human diseases and have been tightly linked to the ageing process and altered stem cell homeostasis. Accordingly, to ensure organismal health, good copies of mtDNA have to be faithfully inherited during cell division, their integrity needs to be maintained over generations and they need to be distributed throughout the mitochondrial network to provide all mitochondrial segments with mtDNA encoded proteins. Astonishingly, it remains poorly understood how cells accomplish these fundamental tasks.

By exploiting the unique advantage that mtDNA can be manipulated in S. cerevisiae, we developed a novel system that for the first time allowed minimally invasive tracking of mtDNA in living cells. Using this system, we have gained unique insights into the cellular principles that govern distribution and inheritance of mtDNA and the maintenance of its integrity. We found that nucleoids are non-randomly spaced within the mitochondrial network and observed the spatio-temporal events involved in mtDNA inheritance. Surprisingly and against the prevailing view, cells deficient in mitochondrial fusion and fission distributed and inherited mtDNA normally, pointing to alternative pathways involved in these processes. We identified such a mechanism, where we observed fission-independent, but F-actin–dependent, mitochondrial tip generation that was linked to the positioning of mtDNA to the newly generated tip. Although mitochondrial fusion and fission were dispensable for mtDNA distribution and inheritance, we showed through a combination of genetics and next-generation sequencing that their absence leads to an accumulation of mitochondrial genomes harboring deleterious structural variations. These data support a role for mitochondrial fusion and fission in maintaining the integrity of the mitochondrial genome by facilitating cleansing of mutant mtDNA copies.

Keywords: *Mitochondria, mitochondrial DNA, mtDNA, S. cerevisiae, Mitochondrial dynamics, Live cell microscopy*

[PS1-2] Systematic Identification and Functional Characterization of Contact Site Resident Proteins in Saccharomyces cerevisiae

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Contact sites are areas of close apposition between organelle membranes that enable the transfer of lipids, metabolites and small molecules in an efficient manner. In recent years, several new membrane contact sites have been described and the tethering proteins that hold the two membranes together have been identified. Despite these advances we are still far from having a deep understanding of the function and regulation of most contact sites. To mechanistically characterize a contact site it is essential to know its entire repertoire of resident proteins yet very few proteins that are enriched in any contact site have, to date, been described. To systematically characterize the proteome of contact sites we have decided to utilize a panel of split fluorescence sensors for a diversity of contact sites in Saccharomyces cerevisiae. With this sensor one part of a fluorophore is fused to the outer membrane of one organelle while the second is fused to another organelle's membranes. If a contact site is present between both membranes, a fluorescent signal is emitted. We have taken sensors for 15 contacts between several organelles such as mitochondria, peroxisomes, lipid droplets, vacuoles, the plasma membrane and the endoplasmic reticulum, and crossed these split-tagged strains with a novel library of mCherry tagged yeast proteins. By analyzing co-localization events we have discovered a large number of new, previously unappreciated, contact site residents. Following up on these proteins, especially those that are conserved to humans, should give us a fresh look at the diversity of functions performed at contact sites and how this affects eukaryotic physiology.

Keywords: *Contact sites, high content screens, proteomics*

[PS1-3] The Protein Architecture of the Yeast Endocytic Machinery Analyzed by FRET

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Clathrin-mediated endocytosis is a principal vesicle trafficking route from the plasma membrane to endosomal compartments. It is essential for membrane homeostasis, recycling of numerous membrane proteins and signaling. To form an endocytic vesicle from a small piece of the plasma membrane dozens of proteins assemble at the endocytic site in a highly coordinated manner. Although the timeline of their assembly is known, their functional arrangement at the endocytic site is still poorly understood. Such information however is critical to mechanistically understand the process of endocytic vesicle formation and its regulation.

Analysis of the protein architecture of the endocytic site is a complicated task given that multiple copies of up to 50 proteins localize in this diffraction-limited spot for a limited

time. Importantly, protein densities at the endocytic site are very well suited for mapping its organization by Förster/fluorescence resonance energy transfer (FRET). Here we show that FRET, a powerful tool to detect proximities between fluorescently labeled proteins separated by less then 10 nm, can be successfully used to analyze the dynamic architecture of the endocytic site.

To map the functional organization of the endocytic machinery we performed a systematic FRET-based proximity screen of 17 conserved endocytic proteins. We obtained multiple highly specific protein proximity pairs, many of them not yet recognized by other (e.g. protein interaction) methods. Further mapping using the clathrin subunits and truncated protein variants as spatial rulers allowed us to draw a detailed map of the yeast endocytic site. Selected protein proximity pairs have been then used to investigate the protein rearrangements occurring during endocytic vesicle invagination by real-time FRET imaging methods. Altogether, our studies show FRET as a highly valuable tool to study protein architectures of molecular machines with the yeast endocytic machinery as an example.

Keywords: *vesicle trafficking, membrane trafficking, endocytosis, live cell imaging, FRET*

[PS1-4] Substrate Transport-Induced Exit from Eisosomes, Ubiquitylation and Endocytosis of Yeast Can1 Arginine Permease

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The yeast plasma membrane contains a wide variety of transporters subject to tight regulation. A common mechanism for inhibiting the function of these proteins is selective sorting into endocytic vesicles, followed by delivery to the vacuole for degradation. This downregulation of yeast transporters is triggered by their ubiquitylation via the HECT-type ubiquitin ligase Rsp5, which interacts with its target proteins via adaptors of the alpha-arrestin family (1-3). Recent studies have shown that stimulation of transporter ubiquitylation can result from direct control of alpha-arrestins according to the metabolic status of the cell or stress conditions. Another condition promoting ubiquitylation and endocytosis of several yeast transporters is transport of their own substrates, avoiding excess uptake. We have now molecularly dissected the mechanisms inducing downregulation of the Can1 transporter during transport of its substrate, arginine. We found that Can1 ubiquitylation requires transition of the protein to an inward-facing conformation. This change of conformation first promotes Can1 segregation out of eisosome microdomains, where the transporter accumulates and is protected against ubiquitylation. It also triggers exposure to the cytosol of a short N-tail sequence necessary for Can1 recognition by the Art1 alpha-arrestin. In addition to this conformational signal, efficient Can1 downregulation requires stimulation of Art1 via the TORC1 kinase complex, itself stimulated upon arginine uptake. Our results define molecular rules likely involved in ubiquitin-dependent endocytosis and degradation of many other transporters of yeast and likely more complex species.

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Keywords: *Membrane transporter, Ubiquitin, Endocytosis*

[PS1-5] Acyl-Coa synthetases activate Fatty Acids on forming Autophagosomes to drive Autophagy

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Macroautophagy is a key intracellular stress response, in which autophagosomes form de novo as double-membrane vesicles and transport enclosed cytoplasmic cargo to vacuoles for degradation. Core autophagy machinery drives poorly defined membrane rearrangements underlying autophagosome biogenesis. Here we identify dedicated long-chain acyl-CoA synthetases (ACS), conserved proteins that activate fatty acids (FA) by coenzyme A-linkage, as part of the autophagy machinery in Saccharomyces cerevisiae. Determined by N-terminally encoded targeting information, ACS localized to nucleated autophagic membranes and progressively accumulated on forming autophagosomes. Cells deficient for local FA activation were severely compromised in autophagy caused by a reduced rate and extended duration of autophagosome formation, and nonproductive autophagosome-vacuole fusion. Our work demonstrates that mechanisms of FA channelling directly drive key stages of autophagy, and provides insights into the fundamental processes of autophagosome biogenesis.

Keywords: *Autophagy, Lipid Metabolism, Proteostasis, Organelle dynamics, Ageing, Quality Control, Fatty acid*

[PS1-6] Global analysis of Atg8 interactome in Saccharomyces cerevisiae using bimolecular fluorescence complementation assay

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Autophagy is an evolutionarily conserved catabolic process for the bulk degradation of cytoplasmic components. Starvation-induced autophagy begins with the formation of double-membrane vesicles, known as autophagosomes, which sequester proteins and organelles and are delivered to the vacuole for degradation. Recently, autophagy has been proved to play crucial roles in numerous human diseases, such as cancer,

neurodegeneration, muscular disorder and so on. Thus, it is important to understand the precise regulatory mechanism of autophagy. Atg8 proteins play multiple roles in the autophagy process. They are responsible for specific recruitment of cargo proteins delivered to the vacuole for degradation and promote autophagosome maturation through interaction with different autophagy regulators or receptors for selective autophagy. Although recent studies have shown that Atg8 is a multifunctional autophagy regulator, genome-wide interactome analysis of Atg8 has not been performed previously. Here, we report the Atg8 interactome in Saccharomyces cerevisiae identified by a genome-wide bimolecular fluorescence complementation assay, a powerful technique for analyzing protein-protein interactions in living cells. Our results provide fundamental insight into the regulation of autophagy and how various cargoes can be targeted for selective autophagy.

Keywords: *Saccharomyces cerevisiae, autophagy, ATG8, Genome-wide, BiFC (bimolecular fluorescence complementation) assay*

[PS1-7] Mitochondrial aggregates as a tool to study the quality control during mitochondrial inheritance in budding yeast

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Budding yeast cells reproduce by asymmetric cell division. Since mitochondria cannot be synthesized de novo, they have to be inherited from the mother cell. We hypothesize that this inheritance is regulated by a quality control mechanism to prevent daughter cells from inheriting damaged mitochondria. In order to analyze what genes are involved in this mechanism we expressed an aggregation-prone protein in mitochondria and analyzed its effect on cellular growth.

We decided to work with TDP-43. In our construct, the TDP-43 gene is fused with a mitochondrial targeting sequence and tagged with GFP. The expression of this protein is regulated by the presence of galactose. We have observed that mitochondrially-targeted TDP-43 forms aggregates inside mitochondria.

A drop-dilution test showed that cells with mtTDP-43-GFP grow poorly under expression conditions compared to an empty vector control. Therefore we decided to perform a high-throughput screen based on growth analysis, specifically synthetic genetic array (SGA). This method enables to analyze the effect of any mutation (i.e. expression of mtTDP-43-GFP) on growth of more than 4500 deletion strains.

For SGA, the query strain containing the studied mutation is crossed with the deletion strain library and after some selection steps, double mutants are obtained. The cells are then transferred on expression medium containing galactose. Expression plates are analyzed and the mean colony size of every strain on the case plate is compared to the control plate.

To confirm the results, the whole experiment was performed twice and only overlapping genes were further studied. In the end, we got a set of 21 confirmed genes. Among them,

there are both genes that rescue the growth defect caused by mtTDP-43-GFP expression and genes that aggravate it. We will now focus on further analysis of these genes and their possible roles in mitochondrial quality control.

Keywords: *mitochondria, budding yeast, inheritance, quality control, aggregates*

[PS1-8] Lipid droplets are central organelles for meiosis II progression during yeast sporulation

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Neutral lipids, predominantly triacylglycerol (TAG) and sterol ester (SE), are stored within the cellular organelle termed lipid droplets (LDs). Although it is believed that the major function of LDs is to supply the cell with energy and membranes, little is known about the cellular events directly involving LDs and their contents. In this study, we provide cytological evidence to reveal that LDs formed direct contacts with the prospore membrane (PSM) that is synthesized de novo during meiosis II to sequester the dividing nuclei in sporulating yeast. Lipidomic analyses revealed that TAG lipolysis drove release of free fatty acids (FAs) at a time correlating well with meiosis II progression and phospholipid remodeling. Through analyzing several aspects of LD physiology, we conclude that proper LD assembly and LD lipolysis driven by TAG lipases are needed for proper cell wall assembly. Yeast cells devoid of LDs were severely defective in PSM growth and organization and disrupted cell wall assembly, thereby producing dead spores or even failing to form spores. These results provide novel insights into the physiology of LDs, linking the organelle directly to a unique membrane morphogenesis process critical for development.

Keywords: *lipid droplet, meiosis II, sporulation, prospore membrane, lipolysis*

[PS1-9] Lipid Droplets: A New Way to “Rejuvenate” the Outer Mitochondrial Membrane

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One of the most widely accepted theories of aging posits that cellular damage by ROS (reactive oxygen species) is a prominent cause of aging. Superoxide originates from a leakage of the mitochondrial electron transport chain. An increased ROS production induces also a change in mitochondrial morphology leading from a tubular network to its fragmentation resulting in blob or donut like mitochondria. If the mitochondrial damage further increases a reversible but deathly pathway is initiated: apoptosis. The apoptotic process at mitochondria includes such milestones as (1) translocation from proteins to mitochondria, (2) mitochondrial fission and cristae disorganization, (3) outer membrane

permeabilization, (4) calcium influx, (5) release of cytochrome c and loss of the mitochondrial membrane potential. Below a certain threshold damaged mitochondria are removed via mitophagy. In the following we want to demonstrate that damaged mitochondria are not necessarily directed for degradation via mitophagy. They can be selectively cleansed from pro- and anti-apoptotic proteins by lipid droplets thereby rejuvenating them. We also characterized a special domain in these proteins that is responsible for both, mitochondrial localization as well as for shuttling them from mitochondria to lipid droplets.

Keywords: *aging, mitochondria, apoptosis, lipid droplets*

[PS1-10] ESCRT and Clathrin-dependent Microautophagy for Lipid Droplet Degradation

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Lipid droplets (LD) are the main organelles for storage of neutral lipids in eukaryotic cells. The core of LDs is filled with two types of neutral lipids: triacylglycerol (TAG) and sterol ester (SE). Recent studies demonstrated that autophagy and LDs are intricately linked. First, LDs are targeted to the vacuole/lysosomes via the autophagic process, termed lipophagy. Next, both biosynthesis and mobilization of the neutral lipids are required for autophagosome biogenesis in Saccharomyces cerevisiae. And our recent study indicated the importance of autophagy in lipid recycling and complex involvement of Atg proteins in LD dynamics (1,2).

Microautophagy refers to a mode of autophagy in which the lysosomal or vacuolar membrane invaginates and directly engulfs target components. The molecular machinery of membrane dynamics driving microautophagy is still elusive. Using immunochemical monitoring of a yeast vacuolar transmembrane protein, Vph1 and Pho8, fused to a fluorescent protein, we obtained evidences showing an induction of microautophagy after diauxic shift in the yeast S. cerevisiae. Components of ESCRT (Endosomal Sorting Complex Required for Transport) machinery were found to be required for this process, and the gateway protein of the machinery, Vps27, was observed to change its localization onto the vacuolar membrane after diauxic shift. We revealed the functional importance of Vps27 interaction with clathrin in this microautophagy that also contributed to uptake of lipid droplets into the vacuole (3). This study sheds light on the molecular mechanism and physiological roles of microautophagy, which does not require conventional core Atg proteins.

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Keywords: *autophagy, vacuole, lipid droplet, ESCRT, clathrin*

[PS1-11] System for Monitoring the Assembly of Eisosomes, Specialized Plasma Membrane-Associated Microcompartments

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Yeast plasma membrane is compartmentalized into a great number of co-existing functional microdomains. Membrane compartment of Can1 (MCC) is one of the best described plasma membrane microdomains in Saccharomyces cerevisiae. Besides Can1, it accumulates other nutrient transporters (Fur4, Tat2) and also tetraspan proteins (of Sur7 and Nce102 families). MCC is organized from the cytosolic side of the plasma membrane by protein complexes called eisosomes. Eisosomes are mostly composed of BAR domain proteins Pil1 and Lsp1. Due to the crescent shape of Pil1 and Lsp1 and their ability to oligomerize, the membrane of MCC is bent into elongated furrows. In S. cerevisiae, Pil1 protein is indispensable for eisosome formation, in contrast to Lsp1. The expression of Pil1 is cell-cycle regulated; new eisosomes are formed only in buds and once formed, eisosomes are reported to be very stable in time. In cells lacking Pil1 protein, the components of MCC are more homogeneously dispersed in the plasma membrane and/or they aggregate with Lsp1 in structures called eisosome remnants.

In this study, we examined the mechanism of eisosome formation using fluorescence microscopy. We controlled the timing of Pil1 expression by cloning Pil1-GFP under galactose-inducible promoter. In pil1Δ cells, galactose induction of Pil1-GFP expression resulted in the de novo formation of eisosomes at the plasma membrane. Moreover, Sur7-mRFP protein translocated from eisosome remnants into newly-formed Pil1-GFP eisosomes. In wild-type cells, the galactose-induced Pil1-GFP was incorporated at the ends of pre-existing Pil1-mRFP eisosomes. Apparently, number and length of eisosomes is determined by the level of Pil1 protein. Bipolar binding of Pil1-GFP onto Pil1-mRFP eisosomes suggested bidirectional character of the eisosome formation.

Keywords: *plasma membrane organization, eisosomes, MCC, fluorescence microscopy*

[PS1-12] Peroxisomal Import of Saccharomyces cerevisiae Proteins Fox2p and Cta1p Is Not Absolutely Dependent on Their PTS1 Signal

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S. cerevisiae peroxisomes are organelles which do not contain DNA and ribosomes, so peroxisomal proteins must be imported from cytosol. There are two known peroxisomal targeting signals: PTS1 consisting of three C-terminal aminoacids, found in a majority of peroxisomal matrix proteins, and amino-terminal PTS2. Import machinery depends on

several membrane and cytosolic proteins called peroxins. Three peroxins act as shuttling receptors between cytosol and peroxisomes: Pex5p which recognizes and transports PTS1 proteins, Pex7p involved in PTS2 pathway and newly discovered Pex9p playing an accessory role in PTS1-dependent import.

There are some peroxisomal proteins which do not contain any PTS sequences, such as acyl-CoA oxidase (AOx), or carnitine acetyltransferase (Cat2p) that have PTS1 but this signal is not necessary for protein transport. Interestingly, these two proteins were shown to interact with the N-terminal region of Pex5p distinct from the C-terminal region involved in PTS1 recognition (containing tetratricopeptide motifs). (Skoneczny M, Lazarow PB., 1998. Mol. Biol. Cell, 9S and Distel B., 2002. J Biol Chem. 277). These results led to a conclusion that Pex5p may be a dual function receptor and play an additional role in the recognition of a yet to be discovered new peroxisomal targeting signal. Apart from AOx and Cat2p, there might be other peroxisomal matrix proteins whose transport is not dependent on PTS1 and PTS2. In our study we found another two proteins of this category, a bifunctional enzyme of peroxisomal β-oxidation (Fox2p) and catalase A (Cta1p). Presented results bring new information about Cta1p and Fox2p peroxisomal targeting and show their complex interactions with Pex5p receptor.

Using GFP tagging of Cta1p and Fox2p in strains expressing different mutated versions of receptor Pex5p, we show possible involvement of the N-terminal region of Pex5p in transport of these two proteins. This region may comprise a recognition site for a hypothetical PTS3 signal.

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Keywords: *peroxisomal import, PTS3 signal, Fox2p, Cta1p, fluorescence microscopy*

[PS1-13] Monitoring the localization and stress-induced dynamics of Sur7 by super-resolution fluorescence microscopy

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It has been widely accepted that the plasma membrane is not a homogeneous gel, but rather a patchwork of co-existing lateral microdomains of specific structural properties and biological functions. This way, various processes, such as signal transduction, nutrient transport or protein trafficking, can be segregated in continuous but distinct membrane regions. In the plasma membrane of S. cerevisiae, one such microdomain is the furrow-like membrane compartment of Can1 (MCC) which is stabilized on the intracellular side by a protein scaffold called eisosome. While the key constituents of the MCC and eisosomes are rather well-documented, their function, dynamics and precise localization within the MCC/eisosome structure are understood only partially. For example, it has been shown that the eisosomal components Pil1 and Lsp1 localize on the negative curvature of the furrow. However, the available data are still somewhat fragmented. The gap in our knowledge

originates in part in the size of the eisosome/MCC which is comparable to the diffraction limit of conventional confocal fluorescence microscopy, i.e. 250-300 nm in diameter. The use of super-resolution fluorescence microscopy techniques, such as SOFI (super-resolution optical fluctuation imaging) and PALM (photoactivated localization microscopy) enabled us to overcome this limit and monitor the localization and stress-induced dynamics of Sur7, a tetraspanning integral plasma membrane protein which localizes on the positive curvature of the eisosome-supported furrow. Since it has been proposed that Sur7 anchors the MCC/eisosome structure to the cell wall, we followed the effect of cell wall digestion. We also investigated the application of the disulfide bond-reducing agent, DTT, on the distribution of Sur7 in time and space.

Keywords: *eisosome, MCC, Sur7, super-resolution microscopy*

[PS2] Telomeres, aging and cell death

[PS2-1] A rudimentary apoptotic pathway protects sporulating yeast from lethal viral accumulation

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As Programmed Cell Death (PCD) acts in opposition to the fitness of the cell executing it, how PCD came about during evolution remains a quandary. A logical hypothesis is that cells capacities for self-killing must have co-opted processes that originally promoted cell survival. We have discovered what might be regarded as a living fossil of PCD that illuminates this hypothesis. Yeast sporulation involves a 5-way cell division, with the 4 meiotic products developing within the remnant of the cell that produced them. We showed that PCD of this remnant occurs as an intrinsic aspect of yeast gametogenesis and is executed through developmentally programmed vacuolar rupture. Intriguingly, undeveloped meiotic nuclei that are frequently swept up in this PCD are subjected to fragmentation of their genomic DNA into nucleosomal ladders, a hallmark of apoptosis. This phenomenon is dependent on NUC1, the yeast homolog of mitochondrial endonuclease G, and is associated with mitochondrial membrane depolarization in the remnant cell that occurs prior to vacuolar rupture. Despite being a prominent feature of apoptosis, the raison d'être for genome fragmentation during mammalian apoptosis remains unknown. Similarly, the underlying role of this DNA fragmentation pathway during yeast meiotic PCD has remained obscure. Yeast possesses numerous viral entities whose persistence requires successful meiotic transmission. We have now determined that NUC1 functions in a rudimentary apoptotic pathway that promotes yeast survival through an innate immune defense against these viral entities. Accumulating L-A and "Killer" dsRNA viruses that are endemic to yeast triggers the release of Nuc1 through an apparent MOMP-like (mitochondrial outer membrane permeabilization) phenomenon, resulting in their downregulation. Failure of Nuc1 to curb the accumulation of the Killer virus causes lethality in mitotic cells. Molecular, biochemical, and genetic analysis confirms that accumulating Killer virus within the cells causes this lethality, illuminating that this rudimentary apoptotic pathway therefore functions in an innate immune capacity. Extension of these findings to meiotic cells confirms that NUC1 prevents the hyper-accumulation of Killer toxin following meiosis. We are interested in the roles this pathway may play for defense of the yeast germline against other genetic parasites such as retrotrasnposons.

Keywords: *Yeast viruses, Sporulation, Endonuclease G, Programmed Cell Death, Evolution, Mitochondria*

[PS2-2] Epigenetic Control Of Aging In Response To Caloric Restriction

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Changes in histone post-translational modifications have been implicated in the aging process. These epigenetic alterations are an attractive model through which environmental signals, such as diet, could be integrated in the cell to regulate its lifespan. However, evidence linking dietary interventions with specific alterations in histone modifications that subsequently affect lifespan remains elusive. We show that deletion of the epigenetic enzyme N-alpha-terminal acetyltransferase Nat4 and loss of its associated histone H4 N-terminal acetylation (N-acH4) extends yeast cellular lifespan. Notably, the longevity induced by deletion of Nat4 is epistatic to the effects of calorie-restriction (CR). Consistent with this, i) Nat4 expression is downregulated and the levels of N-acH4 within chromatin are reduced upon CR, ii) constitutive expression of Nat4 and maintenance of N-acH4 levels reduces the extension of lifespan mediated by CR, and iii) transcriptome analysis indicates that loss of Nat4 largely mimics the effects of CR, especially in the induction of stress-response genes. We further show that nicotinamidase Pnc1, which is typically upregulated under CR, is required for the longevity mediated by Nat4 inactivation. Collectively, these findings establish the epigenetic enzyme Nat4 and H4 N-terminal acetylation as novel regulators of cellular aging that link CR to increased stress resistance and longevity.

Keywords: *Replicative lifespan, Epigenetic modifications, Caloric restriction*

[PS2-3] DNA Circles Cause Nuclear Pore Complex Rearrangements during Yeast Aging

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Saccharomyces cerevisiae confines aging factors in the mother cells during mitosis, in order to ensure the emergence of a rejuvenated and naïve daughter cell. The daughter cell obtains a full replicative potential, while the aging factors in the mother cell cause cellular dysfunction and increased mortality rate. One prominent aging factor in budding yeast is the presence of non-centromeric DNA circles (1). They are formed by homologous recombination during DNA repair. Different studies show that DNA circles interact via SAGA with nuclear pore complexes (NPCs), and that these NPCs cluster together to stay in the mother cell during mitosis (2, 3). However, how these circles contribute to aging is unknown. We wondered whether DNA circle binding through SAGA cause alterations in NPCs and if this could affect cell viability.

We observed that NPCs having a DNA circle anchored lose the basket structure, which normally protrudes into the nucleoplasm. Basket detachment, and specifically loss of Nup60, is required to anchor DNA circles to the NPC in order to confine them to the mother

cell. Basket fixation to the pore or targeting to the DNA circle prevents their interaction with the NPC and releases them from retention in the mother cell. Mutations in SAGA show a same loss of circle retention and are long-lived. We conclude that DNA circles require basketless NPCs to be asymmetrically retained in the aging mother cell. In agreement, we observed a progressive accumulation of basketless NPCs during aging.

These basketless and aged pores fail to recruit the SUMO protease Ulp1. Fixing Ulp1 to the NPC or targeting it to the DNA circle fully destabilizes their interaction with NPCs and their retention in the mother cell, even in the absence of Nup60. The basketless pores fail to recruit Ulp1 via Nup60, thereby preventing desumoytion of targets involved in DNA-NPC interaction. We suggest that its exclusion from NPCs is needed for a stable interaction between DNA circle and NPC, assuring that circles remain confined to the mother cell.

Altogether, DNA circles seem to cause an alteration of the NPC structure during aging, which is needed for their retention. Furthermore, the accumulation of modified NPCs might be a major cause of increased mortality with age.

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Keywords: *aging, Nuclear pore complex, mitosis, DNA, post-translational modification*

[PS2-4] Mitochondria Regulate Yeast Replicative Life Span via RAS-dependent ROS Production from the ER-localized NADPH Oxidase Yno1

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Mitochondrial dysfunction leads to accumulation of reactive oxygen species (ROS) which is associated with cellular dysfunction, disease etiology, and senescence. Here, we used the eukaryotic model Saccharomyces cerevisiae, commonly studied for the aging, to demonstrate how defective mitochondrial function affects yeast replicative life span (RLS). We show that RLS of respiratory-deficient cells decreases significantly, indicating that the maintenance of RLS requires active respiration. Interestingly, the shortening of RLS due to mitochondrial dysfunction was not related to the accumulation of extrachromosomal ribosomal DNA circles, a well-known cause of aging in yeast. Instead, intracellular ROS and oxidatively damaged proteins increased as expected. We show that, while the protein kinase A activity is not elevated, ROS generation in cells with respiratory deficiency relies on the RAS-dependent pathway. ER-localized NADPH oxidase Yno1 also plays a role in producing ROS. Our data suggest that severe defect of mitochondrial respiration accelerates aging by disturbing the stability of proteins in yeast.

Keywords: *Mitochondria, Respiration, TOR, PKA, Ras2, Yno1, ROS*

[PS2-5] Deletion of KlMGA2, a hypoxic regulator, reveals connection between lipid biosynthesis, ROS metabolism and longevity in Kluyveromyces lactis

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Fatty acids (FAs) are essential components of functional cytoplasmic and organelle membranes. A proper FAs membrane composition is critical for membrane function and consequently for cell viability. Indeed, unsaturated and polyunsaturated FAs determine functional membrane properties like fluidity.

Our studies on the respiratory yeast Kluyveromyces lactis indicate that environmental signals, like glucose, oxygen and temperature, regulate FAs biosynthesis [1]. Part of this regulation is mediated by the activity of the hypoxic regulator KlMga2. This transcription factor has homology with Mga2 and Spt23 of Saccharomyces cerevisiae, two proteins associated with ER membrane by their C-terminal region, only maturated in hypoxic condition via a ubiquitin-mediated mechanism, which major target is fatty acid (FA) desaturase gene OLE1 [2].

In S. cerevisiae, single deletions of Mga2 or Spt23 are viable, while the double mutant requires oleate. Deletion of KlMGA2 gene in K. lactis generated a viable strain, although suffering from several deficiencies, as defects of cellular fitness and growth, rag- phenotype (incapacity to grow on high glucose medium when respiration is blocked), alteration in FA membranes composition, defective respiration and altered mitochondrial morphology. All these defects are restored by addition of UFAs (Unsaturated FA) to the medium [3, 4].

In addition to these defects, the absence of KlMGA2 gene caused also increased resistance to oxidative stress and extremely extended longevity. These effects are probably due to the augmentation in catalase activity and KlSOD1-2 expression levels that we observed.

All these results bring to the conclusion of the existence of a connection between hypoxia, fatty acid biosynthesis and ROS metabolism in the yeast Kluyveromyceslactis, with KlMga2 as a direct mediator not only of hypoxic response, but also of oxidative stress response/adaptation.

Work partially funded by MAECI (Direzione Generale per la Promozione del Sistema Paese).

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Keywords: *Kluyveromyces lactis, ROS, longevity, hypoxia, lipid biosynthesis*

[PS2-6] A System For The Purification And Identification Of New Proteins Associated With Telomeres

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Telomere-associated proteins constitute a dynamic complex with two essential functions that ensure genome stability: telomere capping and telomere replication. In order to identify new players for any of these telomere functions, we developed a strategy to purify telomeres in chromatinized form. Our system is based on a linear plasmid with specific sequences that allow purification of this plasmid in native conditions, followed by the release of telomeres by restriction enzymes. I will report the suitability of this system for the purification of telomeric proteins.

We are particularly interested by the identification of telomere-associated proteins after replication fork passage. Actually, whereas telomere replication by telomerase is quite well understood, little is known about what happens when conventional DNA replication machinery passes through capped telomeres. Moreover, re-establishment of capped telomeres after replication fork passage is required, but proteins and molecular mechanisms involved in this process remain elusive. In order to investigate telomeric replication dynamics during replication, we will purify telomeric proteins after replication fork passage. For that purpose, we will control replication fork passage by a conditional removal of the replication origin on the linear plasmid via site-specific recombinases. I will report our current evidence on the feasibility of this strategy.

Keywords: *Telomeres, DNA replication, Mass spectrometry*

[PS2-7] Comparative Analysis of TERs in the Yarrowia Clade

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Ascomycetous yeasts exhibit a great variability in the length and sequence of telomeric repeats. This variability is accompanied by diversification of telomere-binding proteins and ultimately the entire chromosome end-protecting system. Since the sequence of telomeric repeats is specified by the RNA component of telomerase (TER), it is not surprising that TERs themselves are in many ways the most evolutionary divergent components of the

telomerase machinery. However, studies of TERs in various groups of Ascomycota such as Kluyveromyces, Saccharomyces, Candida, Schizosaccharomyces and filamentous fungi were instrumental in the identification of evolutionary conserved sequences and structures in yeast TERs that are essential for telomerase activity. In order to understand the specific evolutionary steps that led to current diversity of yeast telomeric sequences, we analyzed TERs of the Yarrowia clade, which belongs to basal phylogenetical lineages of Saccharomycotina. We have identified putative loci for TER in several Yarrowia clade species and deduced sequences of their telomeric repeats when they were absent from the assembled genomes. Next we have performed comparative analysis of TERs and identified putative conserved elements known from other yeast TERs and a number of sequences that seem to be conserved specifically in this group. To perform a functional analysis of the identified conserved elements, we constructed a deletion mutant of Y. lipolytica (Δter) lacking the entire locus. This mutant rapidly lost the telomeric restriction fragments (TRFs), but after a short crisis recovered by a yet unknown mechanism and exhibited growth indistinguishable from the wild-type strain. Re-introduction of TER on an episomal plasmid restored the TRFs indicating that the chromosomal ends are accessible to telomerase. We are further investigating the role of the conserved elements of TER in telomerase assembly and activity using a combined strategy – using in silico analysis to determine their secondary structure and employing the deletion variants of TER to study their ability to complement the Δter phenotype.

Keywords: *TER, Telomerase, Yarrowia lipolytica*

[PS2-8] Investigating the Role of Tbf1 in Telomere Homeostasis

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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. In Saccharomyces cerevisiae, a large number of genes have been identified that are implicated in telomerase and telomere structure and/or function. However, comprehension of the mechanism of action of these genes and how they relate to other genes is lacking. TBF1 is an essential gene that has been implicated in telomere homeostasis and the DNA damage response, but its precise role still largely remains to be elucidated. It is known that Tbf1 binds T2AG3 repeats within subtelomeric regions, sequences in the majority of snoRNA gene promoters, as well as promoters of some protein-coding genes. Through analysis of novel tbf1 alleles, we discovered that the protein could have a much more direct role in telomere stability. The reverse transcriptase telomerase is responsible for telomere elongation and is constitutively active in S. cerevisiae. When an essential component of telomerase is removed, 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. Introducing a variety of tbf1 mutants into strains that also lack telomerase causes a dramatic change in the rate of senescence. In

addition, point mutations in these tbf1 alleles have allowed us to identify specific residues implicated in the DNA damage response. Characterization of these novel tbf1 alleles allows us to expound on the multiple roles of Tbf1.

Keywords: *TBF1, Telomeres, senescence, DNA damage repair*

[PS2-9] Telomere-binding Proteins of Schizosaccharomyces pombe as a Model for Investigating the Co-evolution of the Components of Yeast Telomeres

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In contrast to most eukaryotes, whose telomeres are composed of conserved and short tandem repeats, ascomycetous yeasts possess extraordinary variability in their telomeric sequences. This divergence is accompanied by a high variability of telomere-binding proteins (TBPs). There are several scenarios how the co-evolution between TBPs and their target sequences might have emerged. We propose the evolution of dsTBPs containing Myb domain(s) may have occurred in two phases. In the first phase, the genome of a common ancestor accumulated several Myb domain - encoding genes and this complexity may have proceeded via neutral evolutionary ratchet. If telomeres in the common ancestor were mammalian-like, they could employed either Tbf1p or Tay1p/Teb1p as the major dsTBP. In the second phase, as the telomeric sequences diverged, these proteins were replaced by more flexible DNA-binding proteins (Rap1p or Taz1p) while retaining their functions in the internal parts of the genome. An excellent model for testing this hypothesis is the fission yeast Schizosaccharomyces pombe. Its telomeric repeats exhibit high degree of heterogeneity. S. pombe cells contain all known types of ds TBPs: Taz1p as the major telomere-binding protein, as well as Rap1p, Teb1p/Tay1p, Tbf1p. However, if the ancestor of S. pombe (and related species) employed Tay1/Teb1-like protein as a dsTBP and only later switched to Taz1p, the question remains which differences in Myb domains of these proteins were relevant in making Taz1p more tolerant to variations in the telomeric sequence. When the Myb domains of TTAGGG-binding proteins (human TRF1 and TRF2 and S. pombe Teb1p) are compared with the Myb domain of Taz1p, the major difference occurs in the helix 2. Using bacterial one-hybrid system, we are experimentally investigating the positions within the Myb domain of Taz1p responsible for its flexibility. Our results show that the engineered Taz1p with Myb1 domain of Teb1p has lost the affinity to telomeric sequences of S. pombe, confirming that Myb domain has the strongest influence on the binding activity of Taz1p. On the other hand, the substitution of Teb1p helix 2 for Taz1p helix 2 does not increase the affinity of Teb1p to S. pombe telomeric sequences, which suggests that the binding flexibility is not determined exclusively by helix 2 motif in Taz1p.

The work is supported by grants from the Slovak Grant Agencies APVV (APVV-15-0022, APVV-14-0253) and VEGA (1/0052/16, 1/0333/15).

Keywords: *telomere, DNA-protein interactions, telomere-binding protein, shelterin, evolution*

[PS2-10] The Budding Yeast N. castellii Efficiently Activates Alternative Telomere Maintenance Mechanisms (ALT) in the Absence of Telomerase

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We have studied the activation of Alternative Lengthening of Telomeres (ALT) in the budding yeast N. castellii (S. castellii) by knocking out the gene encoding the telomerase RNA component (TLC1). Survivors of the telomerase knockout are generated at a high frequency, both in haploid and diploid strains, and can be passaged for 300 generations without exhibiting any major change in colony morphology. In contrast to the Type I and Type II survivors in S. cerevisiae, ALT survivors of this species do not exhibit any major growth crisis at any time-point during this passaging, although a slight variation in growth rate and colony size was observed. Pulsed Field Gel Electrophoresis revealed that the survivors sustain linear chromosomes. To elucidate the molecular mechanism of the ALT pathway employed by this species, we investigated the organization of the chromosome termini by Terminal Restriction Fragment (TRF) analyses. Our results show that telomeres are rapidly shortening after the gene knockout event, and drastic variations in the TRF pattern depict that reorganizations occur within the telomere regions. We furthermore disrupted the EST1 gene, a sub-component of the telomerase holoenzyme, and observed the same cellular and genetic phenotype. We therefore conclude that this ALT mechanism is a general rescue pathway in N. castellii, which is very quickly and efficiently activated when telomerase is disrupted. We are currently isolating and analyzing telomere sequences from these ALT strains to decipher the structure of the telomeres, with the aim to determine what sequences that are forming the functional caps of the chromosomes.

Keywords: *telomere, telomerase, Alternative Lengthening of Telomeres (ALT), chromosome structure, genome maintenance*

[PS3] Growth control and metabolism

[PS3-1] Modified Sterol Composition Influences Kluyveromyces lactis Plasma Membrane Properties

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The plasma membrane properties depend on the composition of lipids and membrane associated proteins. Major lipids of the plasma membrane structure in eukaryotes – sterols, are thought to play a role in ensuring the stability of the lipid bilayer. An extensive literature reports the fundamental contribution of sterols to fluidity, permeability, microdomains formation, protein functionality and membrane activities (Guan et al., 2009; Lingwood and Simons, 2010). In the present work we investigated the influence of the ERG6 gene deletion on the Kluyveromyces lactis plasma membrane properties. ERG6 gene encodes the enzyme Δ24 - sterol methyltransferase catalysing the conversion of zymosterol to fecosterol in the ergosterol biosynthesis. The deletion of KlERG6 gene leads to the accumulation of ergosterol biosynthetic precursors and reduced susceptibility of Klerg6Δ deletion mutant to antifungal azoles, alkali-metal cations and weak organic acids. Using the qRT PCR we observed increased expression of the KlPDR5 and KlPDR12 ABC transporter´s encoding genes in Klerg6Δ cells. The activity of membrane transporters KlPdr5p and KlPdr12p was measured using the fluorescent substrates rhodamine 6G (KlPdr5p), fluorescein diacetate (KlPdr12p) respectively. The results obtained point to the fact that the product of the KlERG6 gene influences both the structure and the function of the K. lactis plasma membrane.

Acknowledgments

This work was supported by grant from the Slovak Grant Agency of Science (VEGA 2/0111/15) and Comenius University (UK/139/2017).

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Keywords: *ERG6 gene, Kluyveromyces lactis, ergosterol*

[PS3-2] Function and Regulation of Sap1 a AAA-ATPase in S. cerevisiae

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AAA-ATPases are involved in diverse functions like protein degradation, DNA recombination, replication & repair, peroxisome biogenesis, membrane fusion, microtubule motors. A well-studied member of the family is CDC48/p97, which functions as a segregase of ubiquitin conjugates for their degradation by the ubiquitin-proteasome system. Here we study functions and regulation of a putative AAA-ATPase Sap1 (Sin1 Associated Protein) in S. cerevisiae, which binds SUMO (Small Ubiquitin-like MOdifier) conjugates. Sap1 also interacts with a pre-mRNA splicing factor Snu66 through a 25 amino acid conserved region termed SIND (Sap1 Interaction Domain). Two isoforms of Sap1 protein (100kDa and 50kDa) are translated from two different mRNAs in a carbon source-dependent manner. The transcriptional corepressor Tup1 controls the expression of Sap1, and the two proteins have distinct localization and likely different function in the cell

Keywords: *AAA-ATPase, Snu66 interactor, Carbon source-dependent expression, protein isoforms*

[PS3-3] Glutamate Greatly Influences Yeast Transcription And Metabolism Causing An Enhanced Growth Phenotype.

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The yeast Saccharomyces cerevisiae can grow on a variety of nitrogen sources. Yeast cells show different responses based on the amount and quality of the nitrogen source. Nitrogen-containing compounds can be classified into preferred and poor nitrogen sources for a given yeast strain. This classification can depend either on how well they are able to sustain its growth or by the ability of the preferred source to cause the repression of genes required for the utilization of other nitrogen sources (Patrice Godard et al., Mol. Cell. Biol. 27.8 (2007): 3065–86).

We investigated growth, metabolism and transcriptional profiles of cells grown in minimal media supplemented with either ammonium or glutamate, both considered good nitrogen sources (Patrice Godard et al., Mol. Cell. Biol. 27.8 (2007): 3065–86). During the exponential phase, cells using glutamate as a nitrogen source show a larger cell volume and a higher average protein and RNA content compared to cells grown in presence of ammonium. In stationary phase, glutamate-supplemented accumulate higher biomass levels. We refer to this behavior as “enhanced growth”.

In glutamate-grown cells, the deepest transcriptional rearrangement takes place after glucose exhaustion. Combined with a metabolomic analysis, these experiments indicate a profound alteration of the metabolism of storage molecules during growth on glutamate.

We are using constraint-based metabolic models (Paolo Cazzaniga et al., Metabolites 4.4 (2014): 1034–87) to gain more insight on the strategy used by the cells to metabolize glutamate. Glucose-limited chemostat cultures at different dilution rates have been used to obtain steady state values for selected fluxes that are being used to constrain genome wide (Benjamin D. Heavner et al., PLOS Comput. Biol. 11.11 (2015): e1004530) or core (Chiara Damiani et al., Nat. Comput. 13.3 (2014): 321–31) yeast metabolic models. Flux balance analysis correctly predicted the optimal growth yield for both simulated nitrogen sources. We are performing reaction deletion analyses to highlight the metabolic routes that are differently employed in the two conditions. In summary, this work provides an integrated computational and experimental approach to define the metabolic strategies used by yeast to cope with alternative nitrogen sources during different phases of the diauxic growth.

Keywords: *Saccharomyces cerevisiae, Metabolism, Systems Biology, Enhanced growth, Transcriptomics, Metabolomics*

[PS3-4] Gsm1, a Novel Zinc Cluster Transcription Factor, Requires the Hap2/3/4/5 Complex for Expression and Plays a Role in Gluconeogenesis

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The budding yeast S. cerevisiae utilizes glycolysis for energy production when grown in glucose medium. Growth on nonfermentable carbon sources leads to changes in expression of many glucose-repressed genes, some of which are involved in respiratory metabolism. Among transcription factors that mediate the switch from fermentative to respiratory metabolism is the Hap2/3/4/5 complex (Hap complex), a master regulator of mitochondrial biogenesis. The Hap complex regulates the expression of its target genes by binding to CCAAT sequence elements in their promoters. Genome-wide transcriptional analyses have revealed that the Hap complex controls the expression of more than one hundred genes, many of which are involved in respiratory metabolism. One potential target of the Hap complex revealed by transcriptional profiling is GSM1, encoding a zinc cluster protein of unknown function. Here we confirm that GSM1 is a target of the Hap complex using lacZ reporter gene analysis. GSM1 has two CCAAT elements located at -287bp and -634bp in its 5'-flanking sequence. A mutation in the CCAAT element at -287bp abolishes GSM1 expression. Gsm1 is a putative transcription factor and genome-wide CHIP analyses revealed many potential in vivo binding sites. We analyzed 25 of them and found that FBP1, LPX1, PCK1, SFC1, and YAT1 require both Gsm1 and Hap4 for optimal expression. FBP1, PCK1, SFC1, and YAT1 play important roles in gluconeogenesis and/or utilization of two-carbon compounds. These four genes are also known to be regulated by transcriptional activator Cat8p, which is also important for the expression of several genes involved in the glyoxylate cycle. Consistent with Gsm1 as a potential regulator in gluconeogenesis, GSM1 overexpression in cells carrying a cat8 mutation not only increase the expression of these genes, but also suppresses the growth defects on lactate medium. We further found that gsm1Δ has little effect on the expression of genes encoding enzymes of the glyoxylate cycle, namely, CIT2, ACO1, ICL1, MLS1, and MDH2. Like GSM1, CAT8 also requires the Hap complex for expression. Taken together, our data indicates that the Hap complex coordinates the

utilization of non-fermentable carbon sources by regulating not only the expression of genes involved in respiratory metabolism directly, but also the expression of genes implicated in gluconeogenesis and utilization of two-carbon compounds, indirectly via transcriptional activators Gsm1 and Cat8.

Keywords: *Gsm1, Gluconeogenesis, Hap2/3/4/5*

[PS3-5] Peroxisomal Metabolism of the Yeast Candida parapsilosis Grown on Hydroxyaromatic Compounds

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The pathogenic yeast Candida parapsilosis utilizes a range of hydroxyaromatic compounds as the only carbon source using either 3-oxoadipate (3OAP) or gentisate pathway (GP). Fumarate and pyruvate are final products of 3-hydroxybenzoate catabolism via GP, while 3OAP converts 4-hydroxybenzoate to succinate and acetyl-CoA [1]. The enzymes of both pathways operate in either cytosol or mitochondria [2]. Several reactions of both pathways can require NADPH, which is regenerated via the oxidative part of the penthose phosphate pathway operating in peroxisomes [3]. When we analyzed transcriptomes of cells grown on hydroxybenzoates by RNA-seq, we identified a number of genes, whose products are involved in peroxisomal functions such as fatty acids oxidation and peroxisomal biogenesis. Indeed, we observed that the assimilation of hydroxybenzoates is accompanied by strong induction of peroxisomes. We also demonstrated that the enzyme activities of catalase were increased significantly on substrates of both GP and 3OAP. However, the activity of isocitrate lyase, a marker from glyoxylate cycle, was induced concomitantly only with GP. Our data suggest that catabolism of hydroxyaromatic compounds is tightly connected with peroxisomes in Candida parapsilosis but the mode of coordination with 3OAP and GP is different.

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Keywords: *3-oxoadipate pathway, gentisate pathway, hydroxybenzoates, peroxisomes*

[PS3-6] Regulatory Roles for Membrane Fluidity in Yeast Flocculation and Hypoxic Growth

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Modulation of lipid metabolism is used to maintain membrane homeostasis and physicochemical membrane properties. We recently described a metabolic engineering approach to experimentally control fatty acid unsaturation that serves as a key regulator of cell membrane fluidity in S. cerevisiae [1]. This approach allows for investigating systematically the physiological effects of changes to fatty acid unsaturation by bypassing the cell's native lipid regulation pathway.

Repression of the sole fatty acid desaturase-encoding gene in S. cerevisiae OLE1 resulted in a surprising phenotype: Flo1p-mediated yeast flocculation which we found is a transcriptional response to low membrane fluidity. Expression of FLO1 is triggered by two ER-resident membrane ordering sensors that are key components in yeast's native lipid regulation pathway and that are activated upon low membrane fluidity. This pathway is also triggered by oxygen-limited growth in wild type cells as Ole1p catalyzed fatty acid unsaturation relies on the availability of molecular oxygen. Transcriptional analysis revealed that not only FLO1 but a set of membrane fluidity-sensitive genes is globally activated as a part of yeast's long term response to hypoxia during fermentation. This shows how the lipid homeostasis machinery of S. cerevisiae is adapted to carry out a broad cellular response to an oxygen-limited environment.

Our yeast strain in which expression of OLE1 is under experimental control also serves as a cellular platform to assay the activity of heterologously expressed stearoyl-CoA desaturases (SCDs). Functional SCDs complement for repressed endogenous OLE1 and abolish flocculation, thus providing for an easy, fast and affordable assay read-out. Based on this assay we expressed putative SCDs from different eukaryotes (fungi, plants, kinetoplastida e.g. Trypanosoma) and confirmed enzymatic SCD activity. This assay also provides a tool to screen for inhibitors of SCDs, which are interesting drug targets in the treatment of a variety of disease states as well as bacterial and parasitic infections in humans.

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Keywords: *Fatty acid unsaturation, membrane fluidity, yeast flocculation, oxygen limited growth, hypoxia, fermentation*

[PS3-7] Extreme Calorie Restriction in Yeast Retentostats Induces Uniform Non-Quiescent Growth Arrest

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Non-dividing Saccharomyces cerevisiae cultures are highly relevant for fundamental and applied studies. However, cultivation conditions in which non-dividing cells retain substantial metabolic activity are lacking. Unlike stationary-phase (SP) batch cultures, the current experimental paradigm for non-dividing yeast cultures, cultivation under extreme calorie restriction (ECR) in retentostat enables non-dividing yeast cells to retain substantial metabolic activity and to prevent rapid cellular deterioration. The use of these tightly controlled yeast retentostats has allowed detailed characterization of the transition from slow to virtually no growth at the level of (quantitative) physiology, transcriptome and proteome.

These prior studies have, however, studied retentostat cultures at the whole culture level, while cells in SP-cultures are known to differentiate into different cell-types. The aim of the current studies was therefore to investigate heterogeneity in yeast cultures transitioning towards cell division arrest under ECR.

Cellular DNA content analyses and visualization of actin structures showed that the majority of cells resided in G1 of the cell cycle. In sharp contrast to starved cultures, including SP cultures, only few cells (< 10%) contained F-actin bodies in continuous cultures, independent of the average culture doubling time. Furthermore, no subpopulations with distinct cellular HSP12 or HSP26 transcript concentrations, genes known to be differentially expressed in different cell types in stationary phase cultures, could be detected under ECR based on smRNA FISH analyses.

These findings show that retentostat cultivation yields highly homogeneous and robust cultures, in which cells most likely survive in an extended G1 phase. Furthermore, it demonstrates that yeast cells exposed to ECR differ from carbon-starved cells and offer a promising experimental model for studying non-dividing, metabolically active, and robust eukaryotic cells.

Keywords: *Saccharomyces cerevisiae, Cell-division arrest, extreme calorie restriction, Heterogeneity, mRNA FISH, Actin structure, Retentostat*

[PS3-8] SOD1 Recovers Mitochondrial Dysfunctions and Changes the Cell Wall Composition in S. cerevisiae Strain Deleted of Porin

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Sod1 (Superoxide Dismutase 1) is the major cytosolic superoxide dismutase responsible for inactivation of superoxide, a free radical that is highly reactive and can cause cellular

damage. Recently, literature data have described others functions for this enzyme in yeast S. cerevisiae glucose metabolism [1]. In a recent work, SOD1 has been proposed to translocate into the nucleo, where acts as a nuclear transcription factor inducing gene expression of ROS-stress related proteins [2]. Related to this achievement we also found a similar modification, upon transformation of the yeast with human SOD1 [3]. In particular we are using a yeast strain (Δpor1) genetically devoid of porin, the most abundant protein of the outer mitochondrial membrane (also called VDAC (Voltage Dependent Anion selective Channel), that exchanges metabolites and ions between cytosol and intermembrane space: porin has a key role in respiratory mitochondrial metabolism since its deletion results in a phenotype unable to grow on not-fermentable carbon source [4].The addition of SOD1 to Δpor1 strain restores the yeast growth on many not fermentable carbon sources, the mitochondrial functionality and the physiological ROS levels. Moreover, the presence of hSOD1 increases the gene expression of β-barrel MOM proteins [3]. By means of a microarray analysis based on a collection representative of the whole S. cerevisiae genome we discovered that the pathways related to the synthesis of components of the cell wall are strongly altered. This observation was confirmed by calcofluor staining of the yeast cell wall. We are now investigating in detail the metabolic relationships between the bioenergetics metabolism delivered in mitochondria and the cell wall formation, and the mechanistic role of the overexpressed SOD1. Many evidence show the implication of SOD1 in metabolism regulation, but this study could explain how SOD1 is involved in mitochondrial metabolism mediated by porin. The connection between SOD1 and mitochondrial metabolism could be relevant in the pathogenesis of neurodegenerative disorders where SOD1 is involved.

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Keywords: *yeast, metabolism, SOD1*

[PS3-9] Metabolic Reprogramming and Stress Resistance Elicited by Glucose Starvation is Coordinated to Extend Chronological Lifespan in Yeast

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Calorie restriction is the only non-genetic means which can effectively extend lifespan and healthspan from yeast to mammals. Although a number of evolutionarily conserved signalling pathways, such as IGF-1 and TOR, are implicated in the regulation of lifespan mediated by calorie restriction, our understanding of the underlying mechanisms is still poor. Using stress response reporters whose expressions are induced by glucose limitation

and starvation, we have screened the whole gene deletion library and identified many genes that are necessary to mount an effective defence against oxidative and heat stresses. They fall into major clusters of signalling pathways, mitochondrial function, r-RNA processing, DNA damage and repair, transcription from RNA polymerase and cell cycle regulation. Subsequent analyses of the 'signalling' mutants not only revealed novel regulators of chronological lifespan (CLS), such as the GSK-3 ortholog Mck1, but also demonstrated that starvation signals transmitted by SNF1/AMPK, PKC1 and those negatively regulated by TOR/PKA (including PAS kinase Rim15, DYRK kinase Yak1 and Mck1) are integrated to enable metabolic reprogramming and the acquisition of stress resistance. The coordination of the metabolic shift to respiration ensures the conversion of toxic fermentation products (ethanol and acetate) to storage carbohydrates, which are used as energy stores for quiescent cells to maintain viability. Our findings suggest that the key to extend lifespan lies with the ability (and the opportunity) to metabolically adapt to different nutrient conditions (metabolic flexibility) and to overcome redox stress associated with metabolic reprogramming to maintain proteostasis.

Keywords: *glucose starvation, chronological lifespan, metabolic reprogramming, stress resistance*

[PS3-10] Schizosaccharomyces Pombe Cardiolipin Synthase Is Part of the Mitochondrial Tandem Protein

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The fission yeast Schizosaccharomyces pombe shares in some aspects more common features with metazoan cells than its distant cousin, Saccharomyces cerevisiae. Unfortunately, the phospholipid metabolic pathways are currently only partially described in S. pombe. That is true also for the biosynthetic pathway leading to the unique mitochondrial anionic phospholipid, cardiolipin. First part of this pathway, biosynthesis of phosphatidylglycerol and its regulation was described 20 years ago [1]. Information is, however, missing about the rest of the pathway.

Here we show that cardiolipin synthase is an essential protein in S. pombe. It is encoded by the ORF SPAC22A12.08c as a C terminal part of a tandem protein. Expression of S. pombe cardiolipin synthase is able to complement deletion of the CRD1 gene of S. cerevisiae and vice versa, S. cerevisiae CRD1 gene complements deletion of S. pombe SPAC22A12.08c. The proper expression levels of cardiolipin synthase and its partner in the tandem protein, mitochondrial hydrolase with unknown function, seem to be regulated at the level of alternate intron splicing. This regulatory mechanism will be discussed.

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Keywords: *phospholipids, cardiolipin, regulation, mitochondria, Schizosaccharomyces pombe*

[PS3-11] Characterization of thermotolerant yeast Kluyveromyces marxianus in the heating condition

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High temperature fermentation is an available technique in the high temperature production of some materials, likely to bioethanol. The brewing yeast, Saccharomyces cerevisiae, is usually used at 25°C to 30°C in ethanol fermentation. If the fermentation, using yeast, is carried out at 45°C to 50°C, some advantages are present, for example, protection of the unsuitable contamination, faster recovery of ethanol, and considerable savings on capital and running costs required by refrigerated temperature control. Thus, the thermotolerant yeast strain of Kluyveromyces marxianus, growing at higher temperature (45°C) than S. cerevisiae, is important in high temperature fermentation. However, the tolerant mechanisms to higher temperature or heat treatment (heat-shock) in that thermotolerant strain are not clearly reported. Here, we analyze the thermotolerant K. marxianus strain for the response to heat-shock and the metabolite flow in the high temperature culture.

The thermotolerant strain IFO 0482 (Institute of Fermentation Osaka) of K. marxianus showed stronger survivals by heat-shock at 50 °C than the S. cerevisiae, unable to grow at high temperature (45°C). The effect of heat-shock on yeast survivals shows that the decrease of the survival rate was slower and the cellular reduction activity assayed by MTT-procedure was more tolerant in K. marxianus than those in S. cerevisiae at 50°C heat-shock. The intracellular trehalose accumulation in K. marxianus was much increase in S. cerevisiae, cultured at 30°C. These results suggest that K. marxianus protects heat-shock induced damages by the accumulation of trehalose. Thus, the trehalose accumulation and the cellular oxidation were assayed for K. marxianus cultured at 45°C. Trehalose was more accumulated at 45°C that at 30°C in K. marxianus, suggesting that trehalose plays an important role in the thermotolerant growth of K. marxianus. Since the cellular oxidation level was higher at 45°C than at 30°C, the activity of anti-oxidative enzymes, catalase and superoxide dismutase (SOD) were assayed. The catalase activity was increased until the stationary phase culture but the SOD activity was only increased in the exponential phase culture at 45°C. These results suggest that K. marxianus is tolerant to culture at high temperature by the activation of anti-oxidative enzyme. Now, we have tried to assay for the gene expression of those enzymes and the other factors (heat-shock proteins etc.) involved in the heat stress.

Keywords: *thermotolerant yeast, high-temperature culture, Kluyveromyces marxianus*

[PS3-12] Quantitative metabolomics of a xylose utilising Saccharomyces cerevisiae strain expressing the Bacteroides thetaiotaomicron xylose isomerase on glucose and xylose

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The yeast Saccharomyces cerevisiae cannot utilize xylose, but the introduction of a few xylose isomerases function well in yeast, overcoming the limitations of the fungal oxido-reductive pathway. In this study, a diploid S. cerevisiae S288c[2n YMX1] strain was constructed expressing the Bacteroides thetaiotaomicron xylA (XI) and the Scheffersomyces stipitis xyl3 (XK) and the changes in the metabolite pools monitored over time. Cultivation on xylose generally resulted in gradual changes in metabolite pool size over time, whereas more dramatic fluctuations were observed with cultivation on glucose due to the diauxic growth pattern. The low G6P and F1,6P levels observed with cultivation on xylose, resulted in the incomplete activation of the Crabtree effect whereas the high PEP levels is indicative of carbon starvation. The high UDP-D-glucose levels with cultivation on xylose indicated that the carbon was channeled towards biomass production. The adenylate and guanylate energy charges were tightly regulated by the cultures, whilst the catabolic- and anabolic-reduction charges fluctuated between metabolic states. This study helped elucidating the metabolite distribution that takes place under Crabtree-positive and Crabtree-negative conditions when cultivating S. cerevisiae on glucose and xylose, respectively.

Keywords: *Metabolomics, Xylose isomerase, Bioethanol, S. cerevisiae*

[PS3-13] Study of Protein Sulfhydration in the Saccharomyces cerevisiae Proteome

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Over the last years, hydrogen sulfide (H2S) has been recognized as a crucial mediator of several physiological processes in mammals and plants. In fact, H2S due to its capacity as a signaling molecule has been proposed as a new gasotransmitter along with nitric oxide and carbon monoxide. H2S is a colorless, malodorous gas, with a high-water solubility that can be cytotoxic in elevated concentrations. This gas flows freely through biologic membranes and can be produced endogenously by bacteria, archaea and eukaryotes. H2S mediates its effects through a protein post-translational modification: cysteine residues of target proteins can suffer a S-sulfhydration in which free sulfhydryl (-SH) from a sulfhydryl donor (H2S) is transferred to a thiol group, forming a covalent persulfide (-SSH). This protein

sulfhydration affects protein function in a positive or negative way. In budding yeast, H2S is produced as secondary metabolite during fermentation processes by the sulfate assimilation pathway, in which sulfate is reduced to sulfide in order to synthetize organic sulfur metabolites. During continuous culture, H2S is capable of cell synchronization in ultradian oscillations and it is conceivable a physiologic role of H2S in glucose metabolism. In this work, we use biotin switch assay in which –SSH groups can be detected by treatment with biotin-pyridyldithio-propionamide (biotin-HPDP) and analyzed by anti-biotin immunoblots. Total protein extracts from yeast cultures treated with sodium hydrosulfide (NaHS), a H2S precursor, were obtained and incubated with methyl methanethiosulfonate (MMTS), a free thiol blocker and then exposed to biotin-HPDP. Also, cells were grown in culture media with or without a nitrogen source, or with different carbon sources. In our results, we observed that a large number of proteins are basally persulfhydrated, after NaHS treatment the number of sulfhydrated proteins increased slightly. In order to identify protein targets of H2S, streptavidin magnetic beads were used to isolated biotinylated proteins and carried out mass spectrometry. Also, our results showed that several enzymes of glycolytic pathway, chaperones and ribosomal proteins are persulfhydrated in fermentative conditions. This post-translational modification is being evaluated in order to elucidate the role of H2S in yeast metabolism. This work was supported by grant CB-238681 from CONACyT (México) and grants IA200315 and IA202217 from PAPIIT-DGAPA, UNAM.

Keywords: *hydrogen sulfide, fermentation, cysteine persulfide*

[PS3-14] A tRNA Modification Pathway Couples Cellular Metabolic State with Cell Cycle Progression

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The decision of a cell to enter the cell cycle can be viewed primarily as a metabolic problem. Cells must appropriately sense available nutrients, and regulate their metabolic outputs, to enter and eventually complete the cell division process. However, specific mechanisms that couple metabolism with cell cycle progression are poorly understood. Our earlier work had suggested that a specific tRNA modification, which incorporates a thiol group on uridines present at the wobble-codon recognizing position (s2-U), acts to sense amino acid availability (Laxman et al Cell 2013). This tRNA modification appears to integrate amino acid homeostasis with cellular translational capacity. In this study, we observe that in the absence of these s2-U tRNA modifications, cells switch their metabolism to an apparent “starvation state”. By combining translational reporter assays, along with targeted, quantitative metabolite analysis, we find that this metabolic reprogramming results in the activation of an amino acid starvation response, and altered accumulation and utilization of amino acids and nucleotides, particularly during nutrient limited conditions. Furthermore, this tRNA modification dependent metabolic switch altered normal cell cycle progression, and also results in hypersensitivity to deoxyribonucleotide depleting agent, hydroxyurea. Collectively, our results suggest that this s2-U tRNA modification pathway play a critical role in overall amino acid sensing, and coupling amino acid and nucleotide homeostasis with entry into the cell cycle.

Keywords: *Metabolism, Cell cycle, tRNA modification, amino acid starvation response, metabolic switch, translational capacity, amino acid and nucleotide homoeostasis*

[PS3-15] Carbon Source- and Mec1-dependent Switching of Ribonucleotide Reductase Catalytic Subunits Promotes Respiratory Growth

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In the yeast, Saccharomyces cerevisiae, fermentation is the preferred process for energy production even under aerobic conditions. However, when carbon source levels are limiting or in the presence of a non-fermentable carbon source, budding yeast are able to reprogram their metabolism. RNR is a conserved holoenzyme that performs the rate limiting step in de novo dNTP synthesis. Mec1 is a budding yeast ATM/ATR protein, best known for its role in mediating replication stress- and DNA damage- checkpoint responses. In this study, we present evidence of the switch between the catalytic subunits Rnr1/Rnr3 as a response of a change in glucose availability or the presence of a non-fermentable carbon source. The Rnr1 downregulation is independent of Mec1 but is mediated by mitochondrial respiration and a change in the intracellular ATP/dATP ratio sensed by an allosteric regulatory site in Rnr1, however the induction of Rnr3 requires a functional Mec1. These observations reveal a functional interplay among Mec1 signaling, RNR regulation and cell metabolism, whereby Mec1 and carbon source dependent induction of Rnr1 or Rnr3 ensures fermentative or respiratory growth, respectively.

Keywords: *Saccharomyces cerevisiae, Ribonucleotide Reductase, Rnr1, Rnr3, ATM/ATR, Mec1, Carbon Source, Respiration, Mitochondria, ATP*

[PS3-16] ylHog1 Is a Key Element for Yarrowia lipolytica Response to Hyperosmotic Stress

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Hyperosmotic stress is one of the main factors that may inhibit the growth of microorganisms, thus many of them developed the comprehensive cell response pathways, that trigger the adaptation to those conditions. The best studied example is high osmolality glycerol (HOG) pathway from Saccharomyces cerevisiae, which results in the production and accumulation of glycerol inside the cells. The pathway consists of osmosensors in the cell membrane and cascade of protein kinases MAPK, where the most important element is kinase Hog1. Unconventional yeast Yarrowia lipolytica synthetizes erythritol under hyperosmotic conditions. The production pathway of this four-carbon polyol is already well described, but little is known about the regulation of this process. BLAST analysis revealed that many proteins from S. cerevisiae HOG pathway have their homologues in Y. lipolytica. In order to determine if HOG pathway might induce also erythritol production, the deletion

of ylHog1 (Y. lipolityca homologous of Hog1) was performed. The resulting strain exhibited significantly higher sensitivity to osmotic stress and impaired erythritol production. These results are a good base for further investigation of HOG pathway in Yarrowia lipolytica.

This work was financed by the Polish National Centre for Research and Development under project LIDER/010/207/L-5/13/NCBR/2014.

Keywords: *hyperosmotic stress, erythritol, HOG pathway*

[PS3-17] How purine starvation is communicated inside the baker's yeast cell

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Yeast strain inability to synthesize particular metabolite and lack of its external supply is example of synthetic starvation. Uracil and leucine starvation leads to lowered stress resistance and shortened cell half life. Adenine depletion, on the other hand, evokes stress (heat, weak acid and oxidative) resistant phenotype, improves desiccation tolerance and does not significantly affect cell half life. Interestingly, adenine starved cells effectively arrest cell cycle in G1 and are insensitive to rapamycin treatment. Therefore we presume, that adenine starvation specific phenotype is communicated in cell by cell signaling pathways and resemble natural starvations (C, N, P).

To dissect how purine starvation is communicated in cells we explored various hypothesis – depletion of crucial intracellular metabolites, rerouting of carbon flow, activation of TOR, PKA signaling systems.

First two hypothesis were rejected as a sole causative agent of increased stress resistance. Results that led to rejection of these hypothesis are demonstrated in poster

Purine starved cells are resistant to environmental stresses and resistance is rapamycine insensitive that points to involvement of cell signaling pathways. Pathways that are responsible for sensing nutritional status are TOR and PKA. Thus we wanted to find out if and which of these pathways - TOR or PKA is responsible for generation of stress resistant phenotype in S. cerevisiae.

To elucidate how purine depletion is perceived in cell we performed tagging and immunostaining of transcription factors as well as qPRC of genes that are regulated by those transcription pathways. Also we truncated transcription factors to see how unability to fully bind to DNA of patricular transcription factor affects purine starvation phenotype. Results so far show that purine starvation phenotype is a intricate interplay of various transcription pathways.

Yeast phenotypes evoked by purine depletion are not only side effects of cultivation in poorly defined medium, but can also serve as a model for several biological phenomena where purine metabolism is distorted.

The results of this research will contribute to understanding of fundamental mechanisms how cell reacts to sudden synthetic starvation and if there are key elements that could be drugable to control eukaryotic parasites with similar metabolic features (auxotrophies).

Keywords: *Saccharomyces cerevisiae, adenine auxotrophy, synthetic starvation, PKA, TOR, nutrient signalling*

[PS3-18] Guanine plays critical role in the development of stress resistance phenotype in adenine auxotrophic strains during purine starvation in budding yeast

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Adenine auxotrophy is a common feature of many baker's yeast laboratory strains. In case of adenine auxotrophy purine de novo biosynthesis is shut down and yeast relies on salvage pathways. Here we expolore how stress resistance is influenced by starvation for purine nucleotides

Purine (guanine and adenine) synthesis in S. cerevisiae shares common metabolite ionosine monophosphate (IMP). IMP conversion to guanine is unidirectional, meaning if cells cannot synthesise adenine de novo, they are unable to synthesize also guanine, thus adenine auxotrophic starvation yields purine starvation, while adding guanine – strictly adenine starvation sets in.

Purine starvation can set in S. cerevisiae adenine auxotrophic strains when these are cultivated in ill-defined media with poor adenine supply. We have identified several adenine starvation effects which leads to elevated stress resistance in various stress conditions. However, up till now, we did not know if these effects are due to general purine starvation or lack of adenine only.

To assess changes caused by purine/adenine starvation yeast strains were grown in media with varying carbon and purine content and exposed to sub lethal oxidative, thermic, weak acid stress. Desiccation was used as an example of severe environmental stress. A panel of stress responsive genes were used to assess the cell state before and after adenine or general purine starvation.

Both, adenine and purine starved cells effectively stopped their cell cycle in G1. In both cases glucose repression was lowered than rapidly growing cells supplemented with necessary agents. Purine starvation increased stress resistance in all sub lethal stresses tested. Thermic and acetate stress resistance was alleviated in strictly adenine starvation (guanine added), but oxidative stress and desiccation stress tolerance was not influenced by addition of guanine thus being strictly adenine starvation dependant.

We presume, that purine starvation effects on yeast stress resistance reflect mechanisms how cell translate nutrient signals into rapid growth or stress resistance phenotype. Difference in stress resistance among adenine and purine starved cells point to the

different nutrient (C or/and N) signalling pathways, which are turned off in concert or separately depending weather cells are purine or adenine starved.

Purine and adenine starvation could provide useful tools to explore general stress resistance signalling mechanisms in yeast cell.

Keywords: *purine metabolism, adenine, guanine, oxidative stress, heat shock, acetate stress, G1 arrest*

[PS3-19] Regulation of NAD+ Homeostasis by N-terminal Acetyltransferase NatB in Saccharomyces cerevisiae

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Nicotinamide adenine dinucleotide (NAD+) is an essential metabolite for cell redox chemistry, metabolism, and signaling. To understand the complex regulation of NAD+ metabolism, we establish a NAD+ intermediate specific reporter system to identify novel NAD+ homeostasis factors. Mutants lacking components of the NatB complex, NAT3 and MDM20, produce and release elevated levels of nicotinamide (NAM) and nicotinic acid (NA). NatB is an Nα-terminal-acetyltransferase responsible for acetylation of the amino terminus of specific Met-retained peptides. Increased NAM and NA levels in the NatB mutants are concomitant with decreased NAD+ levels. The nicotinamide riboside (NR) salvage pathway has an important role converting NR into NAM in NatB mutants, and that increased NAM in these cells largely comes from the vacuolar pool of NR. Our studies suggest autophagy plays a role in trafficking NAD+ intermediates into the vacuole. Supporting this, increased NA/NAM release in NatB mutants is abolished by deleting ATG14, a key autophagy gene. Moreover, intracellular levels of NA/NAM and NR are increased by autophagy inducing nitrogen starvation conditions. Our studies also demonstrate two major NatB downstream pathways contribute to NAD+ homeostasis. NatB mediated acetylation of tropomyosin Tpm1 is required for its proper binding to actin filaments. Overexpression of TPM1 largely suppresses NA/NAM release in NatB mutants, suggesting Tpm1-mediated vesicular trafficking plays a role in NA/NAM homeostasis. Interestingly, genetic manipulations to decrease NA/NAM production and release are not sufficient to restore the NAD+ levels in NatB mutants. This suggests additional NatB targets are responsible for the low NAD+ levels. We show that the levels of Nma1 and Nma2 (nicotinamide mononucleotide adenylyltransferase) are decreased in NatB mutants, and over-expression of Nma1 is sufficient to restore NAD+ levels in NatB mutants. In summary, our results support a model of NatB-mediated regulation of NAD+ homeostasis. Our findings may contribute to understanding the molecular basis and regulation of NAD+ metabolism in higher eukaryotes.

Keywords: *Nicotinamide adenine dinucleotide, Metabolism, N-terminal acetylation*

[PS3-20] Metabolic Control of Lipid Metabolism by Cell Cycle Components

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Understanding the regulation of lipid synthesis and storage has a great interest in biomedical research, since it has often been associated with the onset of metabolic disorders, such as obesity and diabetes type 2. Exacerbated lipid synthesis has also been shown to be extensively associated to development of highly proliferative and aggressive tumors. In cancer research, many studies have focused on investigation of how cell cycle regulators control cell proliferation. As cell proliferation would require an adapted metabolic response, it is reasonable to think in a strict correlation between the regulation of cell cycle and the control of lipid metabolism. By the use of a fluorimetric method that quantifies the neutral lipids stored in the lipid droplets (LD), our group performed a screening using the yeast deletion collection in order to reveal new regulators of lipid metabolism related to the mitotic cell cycle. Interestingly, most of the strains with altered LD content presented an increased content (HLC) rather than lower levels (LLC). Out of 227 mutant strains related to the mitotic cell cycle present in the collection, 12% were classified as HLC strains while only 2% were LLC. As the hallmark of development of lipid disorders are provoked by an increase in lipid synthesis and consequently its accumulation, we decided to focus on the HLC hits. In this group, we identified that the deletion of an E2F-like protein led to accumulation of LDs. The E2F proteins are similar to the SBF complex in yeast, which is responsible for activating the transcription of genes necessary to the transition between the G1 and S phases of the cell cycle. We further investigated the LD phenotype of strains deleted in the other components of the SBF complex and showed they also regulate LD metabolism. These results suggest that the SBF complex is an important mediator between cell cycle and lipid metabolism.

Keywords: *lipid droplets, E2F proteins, cell cycle, Saccharomyces cerevisiae*

[PS3-21] Development of a New Method for Evaluating the Activation of the TORC1 Signaling Pathway in Saccharomyces cerevisiae

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Saccharomyces cerevisiae is the main species responsible for the alcoholic fermentation in the transformation of grape must into wine, being one of the main problems the deficiency of nitrogen sources in the must, which can lead to stuck or sluggish fermentations. A major challenge is to identify the genetic basis underlying the phenotypic variability in nitrogen consumption and metabolism, with emphasis on the study of the TORC1 signaling pathway, given its central role in responding to nitrogen availability and influencing growth and cell metabolism. However, no clear mechanism has been identified by which the pathway is

activated in the presence of nitrogen sources, with the study of allelic diversity appearing as an alternative to identify genes involved in this process. Although there are methods to evaluate the activation of the TORC1 pathway in the presence of nitrogen sources, these are time-consuming, making difficult to analyze large numbers of strains. Therefore, a new microculture method was developed using the luciferase gene as a reporter, which was tested in strains representative of clean lineages described in S. cerevisiae (North American 'NA', Sake 'SA', West African 'WA' and Wine/European 'WE'). For these four strains, the activation of the TORC1 pathway by a proline-to-glutamine upshift was evaluated by traditional methods based on Western blot (Sch9 and Rps6 phosphorylation), and then compared with the new method developed. The results between the methodologies were concordant, verifying that there are phenotypic differences between the different representative strains. This opens the possibility of using this new methodology to detect the molecular basis that determine the differences in activation of the TORC1 pathway in S. cerevisiae through high throughput techniques, like bulked segregant analysis or comparative genomics between a large number of strains.

Keywords: *TORC1 Signaling Pathway, Nitrogen sensing, Natural diversity*

[PS3-22] Genetic and Transcriptional Analysis of Wine Yeast Strains with Divergent Nitrogen Assimilation Profiles

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The yeast assimilable nitrogen (YAN) is one of the most important parameters during the alcoholic fermentation, influencing the yeast growth rate and metabolism. Additionally, YAN deficiency in the grape must is the main source of sluggish or stuck fermentations, resulting in wines with high levels of sugar concentration and possibilities of microbial contamination. In this work, we assessed the nitrogen assimilation profile for a population of 96 segregants, which were derived from the hybrid between two parental strains with different nitrogen requirement and belonging to different phylogenetic clusters, Wine/European (WE) and West African (WA). The segregants were fermented in MS300 and the YAN profile of each strain was analyzed after six days of fermentation, showing a normal distribution of the phenotypes, which is concomitant with the polygenic nature of these traits. Among these segregants, two of them were selected for transcriptomic analysis based on its differences in nitrogen assimilation profiles, specifically in the amino acid consumption. Furthermore, these two segregants were selected due to its ability to complete the fermentation process with low levels of residual sugar (lower than 5 g/L at day 21). The RNAseq analysis revealed 42 differentially express genes in the strain with low amino acid requirements (segregant 692) respect to the strain with high amino acid requirements (segregant 656), with 6 genes repressed and 36 showing overexpression. Among the repressed genes, we found GAP1 gene, a general amino acid transporter, which correlate with the low amino acid requirements observed in the segregant 692. In contrast, LST8 and BTN2 genes were overexpressed in the segregant 692, these genes are involved in TORC1 pathway and arginine assimilation, respectively; correlating with the lower amino acid requirements of this strain. The analysis of the alleles expressed by each segregant,

showed that WA alleles were mainly overexpressed in the segregant with low amino acid requirements. Overall, our result suggests that low nitrogen requirements are related to WA alleles, confirming the trend of the parental strains. Additionally, the results suggest that low amino acid consumption correlate with a high activity of the TORC1 pathway mediated by LST8.

Keywords: *Wine yeasts, Nitrogen, Transcriptional analysis*

[PS3-23] The Role of the Mitochondrial Sco Proteins in the Defense Against Oxidative Stress

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The mitochondrial Sco protein family is highly conserved among prokaryotes and eukaryotes. While the function of some family members as chaperones in the assembly of cytochrome c oxidase (COX) is well established, the role of other members is unknown. In the yeast Saccharomyces cerevisiae, which possesses two members of the Sco protein family, only the deletion of the SCO1 gene leads to respiratory deficiency, whereas lack of SCO2 has no effect on respiration.

Structural and functional studies on the Sco proteins suggest a variety of possible functions including a role in copper homeostasis and redox processes. In addition, the identification of a thioredoxin-like fold further supports a role in oxidative stress defense.

Loss of a putative antioxidant function upon deletion of the SCO genes might be compensated by other proteins. In order to investigate this option, yeast double mutants lacking one of the two SCO genes and concomitantly one of two superoxide dismutase genes (SOD1 or SOD2) were generated and analyzed under oxidative stress conditions. We show that the double mutants sco1/sod1 and sco2/sod1 exhibit a higher sensitivity to oxidative stress as compared to the respective single deletion strains. Our data are in favor of a role of Sco proteins under oxidative stress conditions.

Keywords: *Oxidative stress, Sco proteins, Mitochondria, SOD enzymes*

[PS3-24] Physiological Changes in Saccharomyces cerevisiae in Response to Inositol Depletion

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The polyalcohol myo-inositol is an essential component of membrane lipids and signaling molecules. It can be either taken up from the environment or de novo synthesized from

glucose-6-phosphate. Optimal growth of the yeast Saccharomyces cerevisiae depends on inositol uptake from the medium, whereas its extracellular depletion results in a reduced growth rate and increased storage lipid synthesis.

Although the response of yeast to the lack of inositol has been extensively investigated on the transcriptional level, the reasons for the growth defect are not clear. In this study, we used carbon flux and metabolome analysis to characterise the differences between inositol uptake and synthesis on the metabolic level.

The metabolism of baker's yeast relies on aerobic glycolysis, in which ATP is mainly derived from substrate-level phosphorylation and the cytosolic redox balance is maintained through ethanol excretion. Only a minor part of the energy demand is generated via mitochondrial oxidative phosphorylation. Our results show that the respiratory activity and fluxes through the tricarboxylic acid cycle were even more reduced in a medium lacking inositol. In addition, such conditions resulted in reduced glucose uptake, strongly reduced fluxes through the pyruvate carboxylase reaction and altered concentrations of some metabolites, which are intermediates or end products of glycolysis. In particular, the intracellular concentration of acetyl-CoA was reduced under inositol depleted conditions, a possible explanation for the lower activity of the pyruvate carboxylase.

Furthermore, we found that the growth defect of S. cerevisiae under -inositol conditions was restricted to fermentative growth, whereas no such phenotype was observed during cultivation in media with non-fermentable carbon sources, suggesting a connection between the Crabtree effect and inositol metabolism.

Keywords: *Inositol, Glycolysis, Fermentation, Crabtree effect, Carbon flux and metabolome analysis*

[PS3-25] Characterization of Novel Regulatory Factors Involved in Lipid Storage Metabolism

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In this work, we studied the regulation of lipid metabolism and its connection to other metabolic and regulatory processes in the budding yeast Saccharomyces cerevisiae. In a preliminary study, we investigated two yeast strains with different capacities to store neutral lipids in a genetic screening for quantitative trait loci (QTL), to identify the causal genetic variations that contribute to the increased lipid storage in one of the strains. Like many other phenotypes, the degree of lipid accumulation is a polygenic trait. Consequently, we identified several genes in the segregating sub-population with high lipid content that were almost exclusively the allelic variants from the parent with high lipid content. For the encoded proteins, no roles in lipid metabolism have been described yet and they are

involved in diverse cellular functions, such as regulation of glycogen synthesis, histone deacetylation and mitochondrial translational processes.

Our aim was to characterize these proteins with regard to their molecular functions and their contribution to neutral lipid accumulation. Both knockout as well as allele replacement strains were constructed and analyzed with respect to their lipid composition. Strains with multiple mutations were used to investigate interactions between these genes and to characterize their role in the lipid metabolic and regulatory network of yeast. In addition, strains overexpressing the genes of interest or encoding GFP-fused constructs were used to elucidate their role in storage metabolism.

Keywords: *neutral lipid metabolism, lipid content, quantitative trait loci*

[PS3-26] Directed Evolution of Complex Phenotypic Traits Using Metabolic Models

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Adaptive laboratory evolution is a powerful tool for developing strains with desired traits without genetic engineering or in cases where the desired traits have complex genetic background. However, it is difficult, if not infeasible, to use adaptive evolution to select for traits that do not increase the cellular fitness. To tackle this, we have developed a novel algorithm, EvolveX, for designing a suitable chemical environment (evolution niche) to exert a natural selection pressure on pathways of interest. The resulting evolved strains would then exhibit the desired non-growth associated traits in the target/application niche.

EvolveX achieves this duality through flux simulations using genome-scale metabolic models. The underlying combinatorial optimization problem is tackled by using a combination of mixed-integer linear programming and genetic algorithm. As components of a chemical environment, EvolveX considers substrates as well as effectors (e.g. inhibitors) of metabolic enzymes or regulatory proteins. As a proof of concept, we demonstrate EvolveX by evolving new metabolic traits in Saccharomyces cerevisiae for food applications where GMO are undesired.

Keywords: *Modelling, Evolution, Metabolism*

[PS3-27] Two units of CR complex have antagonistic functions in regulation of FLO11 and biofilm development

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Yeast biofilms are complex differentiated structures, the cells of which are protected from hostile environments, including antifungals, host immune systems and other treatments. In

Saccharomyces cerevisiae, Flo11p adhesin is a key player in formation of biofilm colonies. 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.

Here we show that two protein units of colony remodeling complex (CR), CR1 and CR2, play antagonistic roles in regulation of FLO11 expression and biofilm colony development. While CR1 induces FLO11 expression and promotes formation of biofilm colonies, CR2 represses the FLO11 gene and inhibits formation of biofilm colonies. Our results suggest that the control of the FLO11 gene expression is highly complex and requires detailed investigation.

Keywords: *FLO11, structured colony, gene regulation, yeast biofilm*

[PS3-28] Metabolism of invasive cells of differentiated yeast biofilm colonies

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Wild strains of Saccharomyces cerevisiae, growing on semisolid substrates, are able to form structured biofilm colonies, composed of an aerial part and of invasive pseudohyphae forming the “roots” that attach the colony to the substrate. Wild strains are adapted to hostile natural environments, which is reflected in their colonies, which use several strategies to protect against environmental impacts. Multidrug resistance transporters are active in surface cell layers and protective extracellular matrix is produced in central colony areas. Additionally, production of Flo11p adhesin is indispensable for building the complex architecture of biofilm colonies [1]. Here, we analyzed metabolic properties of pseudohyphal root parts of biofilm colonies using genome wide RNA sequencing and biochemical approaches. We have found that root cells behave like metabolically active cells by activating genes involved in processes such as amino acid and nucleotide metabolism and genes encoding amino acid transporters. Analysis of intracellular amino acids concentrations showed similar levels of amino acids in root cells and in cells from aerial parts of biofilm colonies. These data indicate that amino acids are not accumulated in root cells but are used for proteosynthesis and biomass increase, as documented also by upregulation of expression of many ribosomal genes and genes involved in translation [2]. This work was supported by GACR 13-08605S and Norwegian financial mechanism 2009-14 No. 7F14083.

1. Vachova L, et al. (2011) Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. J Cell Biol. 194: 679-87.

2. Marsikova et al. (2017) Metabolic differentiation of surface and invasive cells of yeast colony biofilms revealed by gene expression profiling, (submitted)

Keywords: *Saccharomyces cerevisiae, yeast biofilm colony, amino acid metabolism, pseudohyphae*

[PS3-29] Metabolic Processes and Regulation of Glycogen Synthesis in Aerial Cells of Differentiated Yeast Biofilm Colonies

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Biofilm formation, connected with the dimorphic switch to filamentous form and the production of protective extracellular matrix, is crucial for yeast colonization, virulence and invasion into substrates. Biofilms are thus very difficult to eliminate and serve as a source of serious systemic infections. We use as our model a Saccharomyces cerevisiae structured biofilm colony that is composed of a surface “aerial” part and agar-invasive “root” part formed by pseudohyphae [1]. We implemented a novel method of separation of cells from aerial and root parts of biofilm colonies to be able to perform genome-wide expression comparison of aerial and root cells. Based on the gene expression data combined with other analyses, we proposed a model of the metabolic pathways that operate in root and aerial cells [2]. We show that environmentally exposed aerial cells are resting cells accumulating glycogen and trehalose and expressing genes involved in glycolysis, stress defense and protein degradation. Expression profiling also provided indications of cell-specific regulations. Subsequent knockout strain analyses identified Gip2p, a regulatory subunit of type 1 protein phosphatase Glc7p, to be essential for glycogen accumulation in aerial cells and Pig1p and Pig2p subunits to have additional minor roles in regulating glycogen synthesis. The work was supported by GACR 13-08605S and Norwegian financial mechanism 2009-14 No. 7F14083.

[1] Vachova L, et al. (2011) Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. J Cell Biol. 194: 679-87.

[2] Marsikova et al. (2017) Metabolic differentiation of surface and invasive cells of yeast colony biofilms revealed by gene expression profiling, (submitted)

Keywords: *Saccharomyces cerevisiae, biofilm, structured colony, metabolism, glycogen synthesis, regulation*

[PS3-30] Nhp6 Proteins Affect Histone Acetylation Landscape By Interfering With Ac-CoA Metabolism

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The Nhp6 A and B are chromatin architectural proteins homologous to mammal HMGB1. In yeast these proteins are encoded by two different genes, NHP6A and NHP6B. These two proteins show, in the core region, 96% of identity, and their N-terminus domain called HMG-box is committed to DNA binding.

Our recent data showed that in nhp6ab mutant there is a decreased histone amount and increased acetylation at the H4 histone lysine 16 (H4K16), particularly evident at the ribosomal loci (rDNA).

It has been highlighted that a link exists between epigenetic alterations and metabolic processes. In particular histone acetylation is dependent on the acetyl-CoA availability. In yeast the acetyl-CoA is produced through many pathways located at cytoplasm, mitochondria and peroxisomes.

We demonstrated that the absence of nhp6ab proteins leads to metabolic genes deregulation causing the overexpression of key enzymes involved in the fatty acid oxidation as POT1. The product of this latter gene a 3-ketoacyl-CoA-thiolase, is responsible for the last step leading to AcCoA biosynthesis from fatty acids. The upregulation of this pathway in a nhp6ab background leads to increased acetyl-CoA avaiability. This metabolite accumulation is related to the histone hyperacetylation phenotype previously observed at rDNA. The observations here reported indicate that both H3 and H4 histones are hyperacetylated in many residues in nhp6ab strain. Moreover, the recovered acetylation state observed in the in nhp6ab/pot1 suggests that inhibition of beta oxidation pathway provides less acetyl-CoA availability thus reducing histone hyperacetylation phenotype.

These findings indicate how the lack of two chromatin proteins affecting metabolic genes expression, is reflected in an altered, genome wide, epigenetic landscape.

Keywords: *nhp6, metabolism, epigenetics, acetylation*

[PS4] Transport, sensing and signalling

[PS4-1] Unraveling the Regulation of the Yeast Ppz1 Phosphatase by the Moonlighting Protein Hal3

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The Ser/Thr protein phosphatase (PPase) Ppz1 is an enzyme related to the ubiquitous type-1 PPases (PP1c) but it is found only in fungi (including pathogenic ones). Ppz1 controls monovalent cation metabolism and it is regulated by an inhibitory subunit, Hal3, which binds to the C-terminal catalytic domain of the phosphatase. Overexpression of Ppz1 is highly toxic for yeast cells, so its de-regulation has been proposed as a target for novel antifungal therapies.

While modulation of PP1c by its many regulatory subunits has been extensively characterized, the manner by which Hal3 controls Ppz1 remains unknown.As a first approach, we have constructed by PCR-prone mutagenesis a library of Ppz1 variants and we have developed a functional assay in search of mutations affecting the binding or/and the inhibitory capacity of Hal3. We have characterized in vivo and in vitro diverse Ppz1 versions carrying single or double amino acid mutations and found that, although in most cases they were clearly refractory to Hal3 inhibition, none of them exhibited significant loss in binding to the inhibitor. Mapping of mutations strengthened the notion that Hal3 does not interact with Ppz1 through its RVxF-like motif (found in most PP1c regulators). In contrast, construction of a 3D model of the C-terminal domain of Ppz1 based on existing related structures revealed that functionally relevant mutations mapped in a conserved acidic and hydrophobic substrate binding channel used by mammalian Inhibitor 2- to regulate PP1c. However, Hal3 does not bind nor inhibit yeast PP1c (Glc7). Therefore, these results indicates that modulation of PP1c and Ppz1 by their regulatory subunits likely differs, although could share some structural features. The differential characteristics would allow insulation of Glc7 and Ppz1 functions in the yeast cell.

Keywords: *protein phosphatases, regulatory subunits, monovalent cation homeostasis, mutagenesis analysis, functional screen*

[PS4-2] The Yeast Mep2 Ammonium Transceptor Physically Interacts With The 14-3-3 Protein Bmh1

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In response to limiting nitrogen levels diploid yeast undergo a dimorphic switch from yeast like growth to pseudohyphal growth. During this morphological change yeast grow as elongated chains of cells away from the colony to search for nutrients. Studies by a number

of groups over many years have established that signal transduction pathways that regulate pseudohyphal growth include the MAP kinase and PKA pathways. An essential but poorly understood component of the regulation of pseudohyphal growth is the Mep2 ammonium importer. Two models of Mep2 function during pseudohyphal growth have been proposed. First, the Mep2 substrate (either ammonium ion, ammonia gas or ammonia gas plus proton) cause changes in cytosolic pH that is sensed by a relevant signal transduction pathway. Second, Mep2 acts as a transceptor that physically interacts with a signalling partner to control pseudohyphal growth. In the transceptor model, Mep2 acts in a way analogous to G protein-coupled receptors undergoing a conformational change during substrate translocation that initiates signaling. We have undertaken a genetic screen to identify potential Mep2 signaling partners and have identified an interaction between Mep2 and the 14-3-3 protein Bmh1. We have confirmed this interaction using western analysis of membrane fractions and importantly established that this interaction is lost when analysing signalling deficient Mep2 mutants. These data and the known role of Bmh1 in signal transduction support the model of Mep2 as an ammonium sensing transceptor.

Keywords: *Pseudohyphal growth, ammonium sensing, transceptor, stress signaling*

[PS4-3] Allelic Variants of the GTR1 Gene, Involved in the TORC1 Signaling Pathway, Affect Nitrogen Transporters Gene Expression and Nitrogen Consumption in Saccharomyces cerevisiae During Alcoholic Fermentation

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The role of TORC1 signaling pathway is to detect nutritional signals, mainly nitrogen, and to coordinate cell growth. Among its targets are genes related to nitrogen transport and metabolism. Therefore, the adaptation of Saccharomyces cerevisiae to changes in the availability of nitrogen imply variations in the activity of this signaling pathway, which are important in the regulation of the expression of its targets genes. Consequently, the study of the TORC1 signaling pathway becomes relevant in certain areas such as wine fermentation, where one of its main problems is the deficiency of nitrogen sources, such as ammonium and amino acids, which leads to sugglish or stuck fermentation. Previous studies have highlighted the GTR1 gene as a candidate to explain differences in nitrogen consumption. The GTR1 gene encodes a GTPase participanting in the EGO complex responsable for stimulating TORC1 in response to the absence of amino acids. Therefore, different alleles of this gene could differentially affect the expression of target genes of TORC1, such as nitrogen transporters and consequently, impact on the nitrogen consumption. In this context, the objective of this work was evaluate the effect of allelic variants of GTR1 on the differential expression of nitrogen transporters and the nitrogen consumption in S. cerevisiae strains during alcoholic fermentation. The GTR1 gene was selected from QTL mapping performed to a tetraparental population, called SGRP-4X, whose parents belong to four distinct clusters: Wine/European, West African, North America and Sake. These allelic variants were validated by reciprocal hemizygosity assay, which were fermented in

synthetic must and determined the nitrogen content by HPLC. Expression of MEP1, MEP2, MEP3, GAP1, AGP1, DIP5, GNP1 and TAT2 by qPCR at three points of the alcoholic fermentation was evaluated. Differences were observed in the consumption of ammonium, arginine, glutamic, serine, threonine, alanine and glutamine when comparing the Wine and West African alleles. In general, the strain with West African allele showed lower expression of the genes evaluate during the first hours of the fermentation process, which could explain the differences in the consumption of the nitrogen sources. In conclusión, the West African allele of GTR1 affects the expression of the nitrogen transporters, possibly by a constant activation of TORC1.

Keywords: *TORC1 pathway, Allelic diversity, Saccharomyces cerevisiae, Alcoholic fermentation, Nitrogen consumption*

[PS4-4] An In Vitro TORC1 Kinase Assay that Recapitulates the Gtr-independent Glutamine-responsive TORC1 Activation Mechanism on Yeast Vacuoles

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Evolutionarily-conserved TOR complex 1 (TORC1) is an essential regulator of cell growth, which responds to nutrients, especially amino acids. TORC1 is activated by amino acids through multiple types of sensing machinery with different specificities for each amino acid in yeast and mammals. In Saccharomyces cerevisiae, leucine, and perhaps other amino acids, was reported to activate TORC1 via the heterodimeric small GTPases Gtr1-Gtr2, the orthologs of the mammalian Rag GTPases. More recently, an alternative Gtr-independent TORC1 activation mechanism that may respond to glutamine was reported, although its molecular detail is not clear.

In studying the nutrient-responsive TORC1 activation mechanism, the lack of an in vitro assay hinders associating particular nutrient compounds with the TORC1 activation status, whereas no in vitro assay that shows nutrient responsiveness has been reported. In this study, we have developed a new in vitro TORC1 kinase assay that reproduces, for the first time, the nutrient-responsive TORC1 activation. This in vitro TORC1 assay recapitulates the previously predicted Gtr-independent glutamine-responsive TORC1 activation mechanism. Using this system, we found that this mechanism specifically responds to L-glutamine, resides on the vacuolar membranes, and involves a previously uncharacterized Vps34-Vps15 phosphatidylinositol 3-kinase complex and the PI(3)P-binding FYVE domain-containing vacuolar protein Pib2. Pib2 interacted with TORC1 in response to L-glutamine on the isolated vacuolar membrane, suggesting that Pib2 is directory involved in the glutamine-responsive mechanism. Thus, the in vitro assay we have developed is proved to be useful for dissecting the glutamine-responsive TORC1 activation mechanism.

Keywords: *TORC1, rapamycin, amino acid, vacuole*

[PS4-5] Modulation of Cation Selectivity of Yeast Plasma Membrane Na+/H+ antiporters by Repositioning a Single Methyl Group

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Potassium is the predominant ion inside the cell and its appropriate concentration is important for many physiological functions. On the other hand, high intracellular concentrations of Na+ (or its analogue Li+) are generally toxic. Among the cation transport systems which participate in ensuring the optimal intracellular level of alkali-metal cations and protons (pH) in most organisms belong Na+/H+ antiporters (CPA/SLC9 family). Their topology consists of 12 transmembrane segments (TMS) and a long hydrophilic C-terminal cytoplasmic domain. Yeast plasma-membrane Na+/H+ antiporters (Nha/Sod subfamily) can recognize all alkali-metal cations as substrates but transport them selectively. For example, the Saccharomyces cerevisiae Nha1 antiporter transports Na+ (Li+) and K+ (Rb+), while the Zygosaccharomyces rouxii Sod2-22 antiporter exports Na+ and Li+, but not K+ or Rb+. To find out the molecular basis of such cation selectivity, we combined protein structure modelling, site-directed mutagenesis, phenotype analysis and cation efflux measurements to localize and characterize the cation selectivity region of the antiporter. A 3D model of the ZrSod2-22 transmembrane domain (based on the X-ray structure of the Escherichia coli NhaA antiporter and primary sequence alignments with homologous yeast antiporters) revealed a close proximity of T141 (TMS4), A179 (TMS5), F180 (TMS5) and V375 (TMS11) forming a phylogenetically highly-conserved (from E. coli to human) hydrophobic filter (hole) in the putative cation pathway's core. A series of mutagenesis experiments verified the model and showed that structural modifications of the filter resulted in altered cation selectivity and transport activity. Strikingly, the single mutation T141S or triple mutations T141S-A179T-V375I in ZrSod2-22 gained a K+ transport capacity. On the contrary, the point mutations A179T or F180S restricted the antiporter’s cation specificity to Li+ and reduced its transport activity, while residues with smaller side chains at these positions (serine or alanine, respectively) preserved a native cation selectivity. Our results indicate that the position of a single methyl group within this part of the protein determines the antiporter’s ability to transport a particular alkali-metal cation. Obtained data help in understanding the structural code, molecular mechanisms of the function and activity regulation in other members of the CPA family.

This work was supported by the project GACR 17-01953S.

Keywords: *yeast, cation/H+ antiporter, cation homeostasis, potassium, salt tolerance*

[PS4-6] The Hsp70 SSB is a new actor in endocytic patch dynamics

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While molecular chaperones are primarily known for their role in protein folding, they have also been shown in recent years to participate in a large variety of cellular functions. Here, we establish for the first time that the Hsp70 SSB plays a novel role in the endocytic process. Endocytosis depends on an extensive network of interacting proteins that has been characterized in detail, but the mechanisms that regulate the spatio-temporal dynamics of the endocytic pathway is not fully understood. We demonstrate here that in the absence of SSB, cells exhibit a severe defect in endocytosis, the endocytic coat proteins assemble in aberrant structures, and the dynamics of the endocytic particles is slower. Our results show that SSB is necessary for the structure and function of several components of the endocytic pathway, in a step necessary for their progression to the late internalization stages. These results define a novel role for Hsp70s, distinct from the classical role in clathrin uncoating. Our study also clearly demonstrates that chaperones have key roles to play in cellular processes beyond basic protein folding, coordinating the assembly and disassembly of large protein complexes such as the endocytic particles.

Keywords: *molecular chaperones, Hsp70, endocytosis, yeast*

[PS4-7] Roles of SPS Pathway Genes (SSY1-PTR3-SSY1) and SIR3 During Cycles of Growth and Starvation in S. cerevisiae Populations With Differing Proportions of Quiescent and Non-Quiescent Cells

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Over its evolutionary history, Saccharomyces cerevisiae has evolved to be well-adapted to fluctuating nutrient availability. In the presence of sufficient nutrients, yeast cells continue to proliferate, but upon starvation haploid yeast cells enter stationary phase and differentiate into non-quiescent (NQ) and quiescent (Q) cells. Q cells survive stress better than NQ cells and show greater viability when nutrient-rich conditions are restored. To investigate the genes that may be involved in the differentiation of Q and NQ cells we serially propagated yeast populations that were enriched for either only Q or only NQ cell types over many repeated growth-starvation cycles. After 30 cycles (equivalent to 300 generations), each enriched population produced a higher proportion of the enriched cell type compared to the starting population, suggestive of adaptive change. We also observed differences in each population's fitness suggesting possible tradeoffs: clones from NQ-lines were better adapted to logarithmic growth, while clones from Q lines were better adapted to starvation. Whole genome sequencing of clones from Q and NQ enriched lines revealed mutations in genes involved in stress response and survival in limiting nutrients (ECM21, RSP5, MSN1, SIR4, IRA2) in both Q and NQ lines, but also differences between the two lines: NQ line clones had recurrent independent mutations affecting the SPS amino acid sensing pathway, while Q line clones had recurrent, independent mutations in SIR3 and FAS1. Our results suggest that both sets of enriched cell-type lines responded to common, as well as distinct selective pressures.

Keywords: *quiescence, experimental evolution, fluctuating environment*

[PS4-8] Lack of 14-3-3 Proteins in Saccharomyces cerevisiae Results in Cell-to-Cell Heterogeneity in the Expression of Pho4-regulated Genes SPL2 and PHO84

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In the yeast Saccharomyces cerevisiae genes involved in high-affinity phosphate uptake (PHO genes) are strongly induced during both phosphate and potassium starvation, indicating a link between phosphate and potassium homeostasis. As the signal transduction processes involved are not completely understood and 14-3-3 proteins are key regulators of signal transduction processes, we investigated the effect of deletion of the 14-3-3 genes BMH1 or BMH2 on gene expression during potassium starvation and focused especially on the expression of genes involved in phosphate uptake.

Genome-wide analysis of the effect of disruption of either BMH1 or BMH2 revealed that the mRNA levels of the PHO genes PHO84 and SPL2 are greatly reduced in the mutant strains compared to the levels in wild type strains. This was especially apparent at standard potassium and phosphate concentrations. Furthermore the promoter of these genes is less active after deletion of BMH1. Microscopic and flow cytometric analysis of cells with GFP-tagged SPL2 showed that disruption of BMH1 resulted in two populations of genetically identical cells, cells expressing the protein and the majority of cells with no detectable expression. Heterogeneity was also observed for the expression of GFP under control of the PHO84 promoter. Upon deletion of PHO80 encoding a regulator of the transcription factor Pho4, the effect of the BMH1 deletion on SPL2 and PHO84 promoter was lost, suggesting that the BMH1 deletion mainly influences processes upstream of the Pho4 transcription factor.

Our data indicate that that yeast cells can be in either of two states, expressing or not expressing genes required for high-affinity phosphate uptake and that 14-3-3 proteins are involved in the process(es) that establish the activation state of the PHO regulon.

Reference

Anemaet IG, van Heusden GPH. Transcriptional response of Saccharomyces cerevisiae to potassium starvation. BMC Genomics 2014;15:1040.

Keywords: *Heterogeneous gene expression, Phosphate uptake, 14-3-3 proteins, Potassium starvation, Ion homeostasis*

[PS4-9] Resistance of Ogataea (Hansenula) polymorpha to Orthovanadate: the Roles of the Phosphate Transport Control and of Mannoprotein Phosphomannosylation

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In order to study mechanisms underlying extreme vanadate resistance of the yeast Ogataea (Hansenula) polymorpha, we performed screenings for genes related to this phenotype by three approaches: (i) selection of O. polymorpha mutants hypersensitive to vanadate, (ii) selection of vanadate resistant mutants in the closely related yeast O. parapolymorpha, whose wild-type resistance is similar to that of S. cerevisiae, and (iii) search for O. polymorpha genes able to increase vanadate resistance of O. parapolymorpha. The first and the third approaches resulted in identification of the gene designated ABV1 (Alcian Blue staining, Vanadate resistance) encoding a Golgi apparatus mannosylphosphate transferase. The second approach revealed the gene designated similar to its S. cerevisiae orthologue PHO87, which codes for the low affinity plasma membrane phosphate transporter. In O. polymorpha deletion of ABV1 decreased vanadate resistance, while in O. parapolymorpha extra copies of this gene increased resistance to vanadate. The PHO87 had a dual effect on the vanadate resistance. Its deletion was able to increase vanadate resistance of O. parapolymorpha indicating that Pho87 is involved in the vanadate uptake as a phosphate transporter. At the same time, protection against vanadate by elevating phosphate concentration in culture medium correlated with expression of this gene, which reflects Pho87 function as an external phosphate sensor. The latter was also confirmed by PHO87 effects on the regulation of promoter of the PHO84 gene encoding a high affinity plasma membrane transporter. Overexpression of ABV1 increased vanadate resistance in O. parapolymorpha even in the absence of PHO87 indicating that some other route(s) of vanadate uptake is affected by protein glycosylation in the secretory pathway. This study was supported by Russian Science Foundation grant 17-14-01092.

Keywords: *vanadate resistance, phosphate transport, external phosphate sensing, glycosylation, Ogataea polymorpha*

[PS4-10] H+-Influx Coupled to Amino Acid Uptake as a Key Signal Stimulating TORC1 in Saccharomyces cerevisiae

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The Target Of Rapamycin Complex 1, TORC1, is a kinase complex conserved from yeast to mammals that controls cell growth by fine-tuning anabolic and catabolic processes. Among the various signals that impinge on TORC1 are amino acids, which modulate TORC1 activity through the conserved Rag family GTPases. In mammalian cells, the intracellular concentration of specific amino acids is sensed by different sensor systems modulating the Guanine nucleotide Exchange Factors (GEF) and GTPase Activating Proteins (GAP) acting on the Rag GTPases. Yet in yeast the upstream regulators of the Rag GTPases (named Gtr1 and Gtr2) responding to amino acids remain poorly known. In the context of our study of the role of TORC1 in the mechanisms promoting the ubiquitylation and endocytosis of the yeast general amino acid transporter, Gap1, we have found that the uptake of ß-alanine by Gap1 activates TORC1 in a manner dependent on the Rag/Gtr GTPases. Yet ß-alanine cannot be used as a nitrogen source and HPLC measurements showed that it is not converted into other amino acids. On the basis of these observations, we explored different hypotheses to

account for TORC1 activation in response to ß-alanine uptake. For instance, the Gap1 transporter could act as a transceptor capable of signaling to TORC1, or the process of ß-alanine transport by itself could somehow activate TORC1. We will present data indicating that it is the influx of protons associated with H+-coupled transport of ß-alanine that stimulates TORC1. We propose that this signal contributes to TORC1 reactivation accompanying exit of cells from various nutritional starvation conditions.

Keywords: *TORC1, Nutrient transporters, Protein trafficking*

[PS4-11] Application of FAP Technology to Study α-Arrestin-mediated Receptor Endocytosis in S. cerevisiae

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G protein-coupled receptors (GPCRs) are activated by many different types of extracellular stimuli, especially various kinds of chemical agonists. Binding of such a ligand to its cognate GPCR activates signal transduction pathways and thereby causes appropriate responses. In yeast, the mating pheromone α-factor binds to and activates signaling emanating from the GPCR Ste2. However, hyperstimulation of Ste2 can cause cell death. Therefore, cells have evolved mechanisms that act at the receptor level to prevent excess signaling. Among the feedback controls responsible for down-regulation of cellular response to GPCR-initiated signaling is removal of the receptor from the plasma membrane (PM) via clathrin-mediated endocytosis. Studies in yeast by others first showed that this internalization process involves marking α-factor-bound Ste2 for internalization by ubiquitinylation mediated by the PM-associated HECT domain-containing ubiquitin ligase Rsp5. However, recent work in our laboratory has shown that α-factor-induced ubiquitinylation of agonist-activated Ste2 by Rsp5 requires either of two intermediary "matchmaker" proteins, the α-arrestins Rod1 and Rog3. PPxY motifs in the C-terminal segments of these endocytic adaptor proteins bind Rsp5 and their N-terminal arrestin fold domains engage Ste2. To provide an assay to dissect the recognition determinants in both the receptor and these α-arrestins required for their productive interaction, we have been applying an innovative new method, called fluorogen-activating protein (FAP) technology, to visualize Ste2 localization. A fluorogen is an organic molecule that is relatively non-fluorescent when free in solution; however, when the fluorogen is bound to a cognate FAP [a human single-chain antibody (scFvs) that has been engineered to bind the fluorogen with high affinity and specificity], the fluorogen-FAP complex is highly fluorescent. To "catch α-arrestins in action", we co-express a GFP-tagged Rog3 allele, Rog3(Δ400-733) (called Rog3Δ400, for short), which lacks its PPxY motif-containing C-terminal tail (and also lacks phosphorylation sites that we think may help them dissociate from their targets) in cells expressing FAP-tagged Ste2 that have been treated with fluorogen. Cells are then exposed to α-factor to examine whether the fluorescent α-arrestin and fluorogen-FAP-Ste2 co-localize at the PM and other aspects of the dynamics of agonist-induced Ste2 endocytosis. Results from such studies will be presented.

Keywords: *G protein-coupled receptor, α-arrestin, endocytosis*

[PS4-12] "The Hog1p kinase regulates the Aft1p transcription factor to control iron accumulation"

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Iron acquisition systems have to be tightly regulated to assure a continuous supply of iron, since it is essential for survival, but simultaneously to prevent iron overload that is toxic to the cells. Our previous work revealed that cells lacking the sphingomyelinase Isc1p exhibit an upregulation of genes involved in iron uptake leading to increased levels of iron (1). In this study, we show that isc1Δ cells also exhibit deregulated iron localization with a decrease in the vacuolar levels. In isc1Δ cells, despite the presence of iron, the low-iron sensing transcription factor Aft1p is dephosphorylated, accumulates in the nucleus and is transcriptionally more active indicating that Aft1p is improper activated. Aft1p activation underlies iron accumulation in isc1Δ cells since deletion of AFT1, or expression of an Aft1p phosphomimetic mutant S210DS224D that favours its nuclear export, abolished iron accumulation. We also show that Aft1p is dephosphorylated and activated under iron replete conditions in cells lacking Hog1p, a kinase with altered activity in isc1Δ cells (2). Co-immunoprecipitation analysis and an in vitro kinase assay revealed that Hog1p interacts with and directly phosphorylates Aft1p. Moreover, Hog1p-Aft1p interaction decreased in isc1Δ cells. We propose that the decrease in Aft1p-Hog1p interaction leads to Aft1p dephosphorylation in isc1Δ cells and, as consequence, to Aft1p activation and iron overload.

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1 Almeida T et al. (2008) Mol Biol Cell 19, 865-76

2 Barbosa AD et al. (2012) Mech Ageing Dev 133, 317-30

Keywords: *Iron, Isc1p, Aft1p, Hog1p, Cell signalling*

[PS4-13] Lysine acetyltransferase NuA4 and acetyl-CoA regulate glucose-deprived stress granule formation in Saccharomyces cerevisiae

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Eukaryotic cells form stress granules under a variety of stresses, however the signaling pathways regulating their formation remain largely unknown. We have determined that

the Saccharomyces cerevisiae lysine acetyltransferase complex NuA4 is required for stress granule formation upon glucose deprivation but not heat stress. Further, the Tip60 complex, the human homolog of the NuA4 complex, is required for stress granule formation in cancer cell lines. Our work suggests that NuA4 is regulating glucose-deprived stress granule dynamics through two distinct pathways – regulating Acetyl-CoA levels and direct acetylation of stress granule proteins. Surprisingly, the impact of NuA4 on glucose-deprived stress granule formation is partially mediated through regulation of acetyl-CoA levels, which are elevated in NuA4 mutants. While elevated acetyl-CoA levels suppress the formation of glucose-deprived stress granules, decreased acetyl-CoA levels enhance stress granule formation upon glucose deprivation. We found that NuA4 modulates acetyl-CoA levels through the regulation of Acetyl-CoA Carboxylase Acc1. We have also determined that NuA4-dependent lysine acetylation of the core stress granule component poly-A-binding protein Pab1 at lysine 131 contributes to the formation of glucose-deprivation stress granule assembly. Altogether this work establishes that NuA4 regulates the formation of glucose-deprived stress granules through acetylation of Pab1 and through regulation of Acc1 and the metabolite acetyl-CoA.

Keywords: *Stress Granules, Lysine acetyltransferase, NuA4, Pab1, glucose deprivation, Acc1, acetyl-Coa*

[PS4-14] Characterizing Vacuolar Protein Sorting Pathways in the Yeast Pichia pastoris (Komagataella spp)

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Besides being frequently used as host for production of recombinant secretory proteins, the yeast Pichia pastoris (syn. Komagataella spp) is also used as a model system to study the secretory path¬way. This is due to the observation that in contrast to the commonly used yeast model Saccharomyces cerevi¬siae, where the Golgi is distributed throughout the entire cell, P. pastoris forms ordered Golgi stacks similar to the mammalian Golgi, which are located next to discrete transitional ER sites.

The further steps of vesicular transport in P. pastoris, in particular the protein sorting pathways towards the vacuole, were not well characterized so far. Thus, we set out to identify genes involved in these vesicular transport steps and to generate mutants in the vacuolar protein sorting pathways. In these strains, we investigated the changes in secretion of native vacuolar and recombinant secretory fluorescent reporter proteins as well as their intracellular localization. Vacuolar morphology was assessed by fluorescence microscopy. Also in P. pastoris, vacuolar targeting occurs through the well-described CPY and ALP pathways which are named after one of their transported cargo proteins (carboxypeptidase Y and alkaline phosphatase, respectively). Disruption of either of the two

Golgi-to-vacuole pathways can be complemented by the other pathway. Interestingly, several genes that are non-essential in S. cerevisiae proved to be essential in P. pastoris, indicating differences between the two yeasts. Furthermore, synthetic lethality was observed for ALP and CPY components, as well as for genes involved in endosome-to-vacuole fusion and late steps of secretory transport. Furthermore, the impact of the vps mutant strains on secretion of heterologous proteins was investigated. Approaches how to efficiently adapt the host cell's secretion capacity will be presented, which confirm that impairment of vacuolar protein sorting is an effective means of enhancing secretion of heterologous proteins.

Taken together, these studies allowed us to gain comprehensive insights into the function and regulation of vacuolar and endosomal protein sorting pathways in P. pastoris. Apart from contributing to our understanding of intracellular transport, these strains proved be valuable tools for production of recombinant secretory proteins.

Keywords: *Pichia pastoris, protein secretion, vacuolar protein sorting, vacuolar transport*

[PS4-15] Ypq2, the Yeast Ortholog of Human Lysosomal PQLC2 Cationic Amino Acid Exporter, is a high-affinity transporter catalyzing passive arginine transport across the vacuolar membrane

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Ypq1, -2, -3 are highly similar transporters of the PQ-loop family known to be localized at the yeast vacuolar membrane (1). Several previous observations – e.g. transcriptional repression of YPQ3 under lysine excess conditions (1), high turnover of Ypq1 upon lysine starvation (2), resistance of ypq2 mutant to canavanine (a toxic analog of arginine) (1), reduced ATP-dependent uptake of basic amino acids in vacuolar membrane vesicles isolated from ypq mutant (3) - suggest a role of Ypq proteins in transport of cationic amino acids (arginine, lysine, histidine) across the vacuolar membrane. PQLC2 is a mammalian ortholog of the yeast Ypq proteins. Biochemical characterization of PQLC2 expressed to the cell surface of oocytes showed that the protein is a lysosomal exporter of basic amino acids. Furthermore, PQLC2 was found to play a central role in therapy against cystinosis, a lysosomal storage disease, by mediating export from the lysosome of a drug derivate resembling lysine (1). As the yeast vacuole is known to accumulate high amounts of basic amino acids including arginine, we sought to further investigate the role of Ypq proteins in arginine transport across the vacuolar membrane. We have implemented the previously described methods used for isolating intact vacuoles (still containing their sap) and we set up conditions for measuring the activity of passive and active amino acid transporters. We will present data showing that Ypq2 is a high-affinity transporter catalyzing passive transport of arginine and histidine across the vacuolar membrane. Work is in progress to assess the role of Ypq2 in vacuolar export of arginine under normal and starvation conditions. As PQLC2 expressed in yeast is able to complement the phenotype of an ypq2 mutant (1), we hope that our study of Ypq2 will shed more light on the physiological role of PQLC2 in human cells.

Keywords: *vacuole, cationic amino acid transporter, Ypq2, PQLC2*

[PS4-16] Feedback Inhibition of the Rag GTPase GAP Complex Lst4-Lst7 Safeguards TORC1 from Hyperactivation by Amino Acid Signals

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The target of rapamycin complex 1 (TORC1), a conserved serine/threonine kinase complex localized at the vacuolar membrane in yeast, is a major cell growth controller. It integrates a variety of stimuli including nutrients such as amino acids and modulates multiple catabolic and anabolic processes accordingly. Amino acids regulate TORC1 via the heterodimeric Rag GTPases Gtr1-Gtr2, which themselves integrate amino acid signals via their regulators that include GTP exchange factors (GEFs) and GTPase activating proteins (GAPs). One such regulatory module is the Lst4-Lst7 complex that functions as a GAP for Gtr2. This complex exhibits a dynamic subcellular distribution: when cells are starved for amino acids, it localizes to the vacuolar surface; upon amino acid re-feeding, however, it stimulates Gtr2 (and consequently TORC1) and is then rapidly dispersed form the vacuolar membrane. Interestingly, the crystal structure of the N-terminal region of Lst4 revealed the presence of a split DENN domain that is involved in the interaction with Lst7. The intra-DENN loop stretches over 200 amino acids and is not required for the interaction of Lst4 with Lst7. Here, we show that this intra-DENN loop is in fact necessary for anchoring the entire Lst4-Lst7 complex to the vacuolar membrane. We also provide evidence, both in vivo and in vitro, that Lst4 is a target of TORC1, which directly phosphorylates several residues specifically within the intra-DENN loop. In addition, we show that the phosphorylation status of the intra-DENN loop determines the localization of the Lst4-Lst7 complex rather than its GAP activity towards Gtr2. Intriguingly, expression of an Lst4 variant that cannot be phosphorylated by TORC1 mediates hyperactivation of TORC1 and causes growth defects when cells are grown on a poor nitrogen source, while an Lst4 variant mimicking the TORC1-phosphorylated state cannot reach the vacuolar membrane and compromises TORC1 activation by amino acids. Combined, our data support a model in which TORC1 feedback regulates its own activation by displacing Lst4. This mechanism prevents TORC1 hyperactivation in response to amino acids and therefore lends support for the emerging view that TORC1 is a homeostatic controller of growth.

Keywords: *target of rapamycin complex 1 (TORC1), Rag GTPases, Lst4-Lst7 GAP complex, amino acid signaling, growth control*

[PS4-17] Function of Wsc Family Proteins as a Methanol-sensing Machinery in the Methylotrophic Yeast Pichia pastoris

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Methylotrophic yeasts are capable of using methanol as the sole carbon and energy sources. In these yeasts, many genes involved in methanol metabolism and robust peroxisome proliferation are induced during growth on methanol. Several transcriptional factors for methanol-inducible genes have been identified and characterized from methylotrophic yeasts. However, the mechanism of how cells sense the presence and concentration of methanol, and how cells transmit the signal through intracellular signaling pathway to transcriptional factors remain as an open question. Wsc family proteins are plasma membrane spanning sensor proteins conserved from yeasts to mammalian cells. We investigated the functional roles of Wsc family proteins in Pichia pastoris and found that PpWsc1 and PpWsc3 function as a methanol-sensing machinery during growth on methanol (1). In the Ppwsc1Δ strain, expression levels of methanol-inducible gene were significantly lower than those in the wild-type strain. In addition, we showed that expression levels in the double gene disruption strain of Wsc family proteins (Ppwsc1ΔPpwsc3Δ) were lower than those in the Ppwsc1Δ strain. These results indicated that PpWsc1 and PpWsc3 are involved in regulation of methanol-inducible gene expression. Further analyses revealed that PpWsc1 responds to a lower range of methanol concentrations than PpWsc3 and PpWsc1 also functions during high temperature stress, but senses methanol as a signal that is distinct from high-temperature stress. Moreover, we showed that downstream factors of Wsc family protein are also involved in regulation of methanol-inducible gene expression.

Reference

1) Ohsawa et al., Mol. Microbiol. 104: 349-363 (2017).

Keywords: *Methylotrophic yeast, Wsc family protein, Methanol-inducible gene expression, Cell wall integrity pathway*

[PS4-18] Role of glycerol-H+ symporters of non-conventional yeast species in the osmotolerance

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Microorganisms produce different osmoprotectants in order to adapt to environmental changes such as changes in water activity. Glycerol plays this role in most yeast species and is produced at high quantities under hyperosmotic conditions. The osmotolerant yeast species (e. g. Zygosaccharomyces rouxii, Debaryomyces hansenii) are distinguished by a more efficient glycerol uptake system, which helps them to reach necessary intracellular concentration with relatively low glycerol production. Saccharomyces cerevisiae possesses two systems for glycerol transport. First of them is Fps1, a plasma-membrane channel that is required for a quick release of glycerol. Second transporter, Stl1, mediates active uptake of glycerol in symport with protons and is repressed and inactivated by glucose in S. cerevisiae. The regulation of glycerol transport in the wine-making yeast species (e.g. Saccharomyces kudriavzevii, bayanus; Dekkera bruxellensis), which are exposed both to

hyperosmotic stress and glucose presence during the fermentation processes, is probably even more complex.

We have identified putative orthologues of the S. cerevisiae STL1 in genomes of S. kudriavzevii (SkSTL1), S. bayanus (SbSTL1) and in the genome of D. bruxellensis (DbSTL1 and DbSTL2). All STL genes were cloned and expressed in a series of mutant S. cerevisiae strains lacking different combinations of genes involved in osmotolerance. The presence of all these proteins improves growth of S. cerevisiae hog1Δ stl1Δ upon osmotic stress (except DbStl1) and upon conditions where glycerol was used as a source of carbon. Only cells producing DbStl1 did not profit from its presence upon osmotic stress. The possible explanation of this behaviour is the lower efficiency of DbStl1 in glycerol transport.

Supported by GA CR 15-03708S.

Keywords: *glycerol transporters, hyperosmotic stress, non-conventional yeasts*

[PS4-19] The Role of the Yeast Erv14 Protein in the Export of Alkali Metal Cations from Cells

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Alkali-metal-cation homeostasis is crucial to all living organisms, including the yeast Saccharomyces cerevisiae. S. cerevisiae cells possess several transporters, which together ensure maintaining of proper alkali-metal-cation concentrations both in the cytoplasm and in the organelles. There are two main alkali-metal-cation exporters at the plasma membrane, the Na+,K+/H+ antiporter Nha1 and the P-type ATPase Ena1. Erv14 COPII cargo receptor was shown to interact with the Nha1 antiporter (1,2). Erv14 binds the transmembrane domains of Nha1 and its lack results in the retention of Nha1 in the ER and in increased cell sensitivity to toxic sodium cations (2).

In this work, we studied the function and localization of Nha1-family antiporters from various yeast species in S. cerevisiae cells either lacking or possessing Erv14. Nha1 homologues included in the study significantly differ in the lengths of their hydrophilic C-termini and our results suggest that the importance of Erv14 in the delivery of Nha1-family proteins to the plasma membrane is proportional to antiporters´ structure, as the lack of Erv14 mainly affected the localization of Nha1 homologues with long C-terminal parts. Furthermore, we studied the effect of the lack of Erv14 protein on the function and localization of S. cerevisae Ena1 ATPase and we found that Ena1 is properly localized in the plasma membrane of cells lacking the Erv14 protein.

(1) Herzig Y, Sharpe HJ, Elbaz Y, et al. (2012) PLoS Biol 10:e1001329.

(2) Rosas-Santiago P, Zimmermannova O, Vera-Estrella R, et al. (2016) Biochim Biophys Acta 1858:67-74

This work was supported by a GACR grant 17-01953S.

Keywords: *alkali-metal-cation homeostasis, Nha1 antiporter, Erv14 cargo receptor*

[PS4-20] Variability of the Main K+-Importer Trk in Pathogenic Candida Species

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Several tens of Candida species belong to the human pathogens capable of inducing life-threatening infections. Virulence of single Candida species depends among others on its ability to collect sufficient amount of various nutrients. Potassium is the main intracellular cation, for which Candida and host cells compete, because the adequately high intracellular K+ concentration is essential for control of many fundamental biological processes such as the regulation of cell volume, intracellular pH and membrane potential.

In general, yeast cells use three types of potassium importers differing in their transport mechanisms (Trk uniporters, Hak H+-K+ symporters and Acu ATPases). We identified the genes encoding putative K+-uptake systems in genomes of nine Candida species, which differ in their ability to grow on limiting low concentration of KCl and also in the number of genes encoding potassium importers. Nevertheless, we did not find a direct proportionality between the number of putative transporters and the ability to grow on extremely low K+ concentrations.

All studied Candida species have at least one putative Trk1 transporter. We compared Trk systems from three clinically important Candida species upon heterologous expression in S. cerevisiae Δtrk1Δtrk2 strain lacking its own potassium transporters.

Single Trk systems differ in their ability to provide S. cerevisiae Δtrk1Δtrk2 cells with sufficient amount of K+ for growth and proliferation, while the most effective is the Trk1 from the major pathogen C. albicans, less effective is Trk1 from C. glabrata, and the worst is Trk1 from C. krusei. We also identified a second putative gene for Trk system in the genome of C. krusei, which, however, seems to be non-functional upon heterologous expression in S. cerevisiae.

The presence of functional Trk system is also crucial for resistance to toxic Li+ because an active uptake of K+ via specific high-affinity transporters prevents non-specific influx of Li+ into the cell. Li+ resistance of S. cerevisiae Δtrk1Δtrk2 cells is mostly increased upon expression of CgTrk1, less by CaTrk1 and the least by CkTrk1.

The following functional characterization and stuructural analysis of Trk systems from various yeast species would help to elucidate their key parameters such as transport mechanism or activity regulation.

This work was supported by GA CR 16-03398S project.

Keywords: *Candida, Trk, potassium, transport*

[PS4-21] Contribution of Phosphatidylserine to Protein Kinase C Signaling in Saccharomyces cerevisiae

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Protein kinase C (PKC) was identified as a Ca2+-dependent protein kinase in mammalian cells. To date, mammalian PKCs are classified into three groups according to their domain structures and their activating factors. Diacylglycerol (DAG) binds to the C1 domain of PKC, which leads to activation of PKC. In the budding yeast S. cerevisiae, PKC1 is a sole gene encoding PKC. Although yeast Pkc1 contains the C1 domain, Pkc1 is not activated by DAG. In addition, the physical interaction between the C1 domain of yeast Pkc1 and lipids that constitute membranes, including DAG, has not yet been characterized. On the other hand, it has been reported that the C1 domain of Pkc1 bound to the small GTPase Rho1. Rho1 as well as Pkc1 is an upstream module for the Mpk1 mitogen-activated protein (MAP) kinase cascade. In the present study, we show that phosphatidylserine (PS) physically interacts with the C1 domain of Pkc1, and PS plays a pivotal role in the physical interaction between Rho1 and the C1 domain of Pkc1. CHO1 encodes a PS synthase, and the stress-induced activation of Pkc1-Mpk1 MAP kinase cascade was abolished in a cho1 mutant. Bud tip localization of Pkc1 was perturbed in cho1 cells, and the physical interaction of Rho1 and Pkc1 was also impaired in this mutant. Furthermore, repolarization of actin patches under heat shock-stressed condition, the regulation of which the Pkc1-Mpk1 MAP kinase cascade plays a role, was impaired in cho1 cells. These results suggest that PS is involved in the Pkc1 signaling through regulating the localization of Pkc1 as well as physical interaction between Rho1 and Pkc1.

Keywords: *Pkc1, phosphatidylserine, Rho1*

[PS4-22] Kch1 and Kch2 Membrane Proteins Participate in Membrane Potential and Monovalent Cation Homeostasis Maintenance in Saccharomyces cerevisiae but not in Candida albicans

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The maintenance of intracellular ion homeostasis is a crucial attribute for any living cell. The model yeast Saccharomyces cerevisiae is a key organism for studying transport systems and ion levels regulation in eukaryotic cells. Our work is focused on physiological characterization of plasma-membrane proteins Kch1 and Kch2 homologous to animal voltage-gated Ca2+ channels, which were shown to be essential for the K+-dependent activation of a high-affinity Ca2+ influx system in S. cerevisiae1. To search for their role in the maintenance of alkali-metal-cation homeostasis, strains with deletions of KCH1 and/or KCH2 genes in BY4741 background and its derivatives lacking main potassium uptake system (Trk1, Trk2) were constructed. We tested the ability of these strains to grow in potassium-limited conditions, and their tolerance to salts and extreme external pH. To find

out other contributions of Kch1/Kch2 proteins to cell physiology, relative membrane potential, cell volume and intracellular pH were measured.

S. cerevisiae strain lacking both KCH genes grew better on media with low K+, its plasma membrane was relatively hyperpolarized, and cell size was significantly smaller compared to the wild-type cells. In addition, this strain exhibited altered growth in the presence of monovalent cations. The growth at different external pH and the intracellular pH remained unaffected in the strain lacking both KCH genes. Nevertheless, the overexpression of KCH1 gene resulted in higher intracellular pH in wild-type cells growing in media with elevated potassium concentrations. All phenotypes caused by the deletion of KCH genes were associated with the presence of potassium importers Trk1 and Trk2. We also examined the importance of Kch homologue in the maintenance of cation homeostasis in Candida albicans. However, no differences were observed between the wild-type and kch1/kch1 homozygous mutant in tested phenotypes. All our data demonstrate that fungal-specific Kch proteins significantly contribute to the maintenance of optimal cation homeostasis and membrane potential in S. cerevisiae but not in C. albicans.

This work was supported by GACR (16-03398S).

Reference

1 Stefan, C. P. et al. Eukaryotic Cell 12, (2013).

Keywords: *Kch1, Kch2, monovalent cation homeostasis, plasma-membrane potential, intracellular pH, Saccharomyces cerevisiae, Candida albicans*

[PS4-23] Calcium Mediated Response to Cd2+ and Cu2+ Stress in Saccharomyces cerevisiae Cells

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Aim: The immediate response to changes in the environment is essential for cell survival under stress conditions. Ca2+ ions are often involved in the cellular response to various stressors, being used as second messenger by virtually all eukaryotic cells.To understand the ways in which cells sense the presence of toxic concentrations of metals in the environment, the involvement of Ca2+ in the response to heavy metal stress was investigated in Saccharomyces cerevisiae cells.

Experimental design: To see if the cell response to excess metals was Ca2+-dependent, the modifications in cytosolic Ca2+ were recorded in transgenic yeast mutants expressing the Ca2+-dependent photoprotein, aequorin. The involvement of Ca2+ channels and exchangers in metal tolerance was investigated, along with the Ca2+ signaling profiles of yeast cells with defects in metal cellular transport.

Results: 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+. 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 Cd2+ and Cu2+ are discussed.

Conclusions:The tolerance to high Cd2+ often correlated with sharp Cd2+-induced cytosolic Ca2+ pulses, while the Cd2+ sensitivity was accompanied by the incapacity to rapidly restore the low cytosolic Ca2+.On the other hand, the Ca2+-mediated response to surplus Cu2+ depended on the cell possibility to restrict the reduction to- and the accumulation of Cu+.

Keywords: *heavy metal stress, calcium signaling, aequorin, Saccharomyces cerevisiae*

[PS4-24] Regulation of Entry into Meiosis in Budding Yeast

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In budding yeast Saccharomyces cerevisiae, a binary cell fate decision is made based on the mating type status of the cell and the availability of nutrients in the environment. When nutrients including glucose and nitrogen compounds are depleted in the growth medium, diploid yeast cell undergoes a highly conserved differentiation program known as gametogenesis or sporulation. During sporulation, diploid cells undergo a single round of DNA replication followed by two consecutive nuclear divisions, i.e. meiosis, to generate progeny with half the number of chromosomes in the parent cell. The cell's decision to enter meiosis is tightly regulated and the decision is only made when IME1, the master regulator of entry into sporulation is activated. IME1 has one of the longest promoters in budding yeast (>2 kb) and consists of many regulatory elements. Importantly, the IME1 promoter functions as a signal integrator where all the mating type and nutrient signals converge to regulate the expression of IME1. Transcription of a long non-coding RNA ensures that cells with haploid mating type cannot induce IME1. In addition, glucose and nitrogen compounds in the growth medium activate the Ras/PKA and TORC1 pathways respectively to inhibit IME1 expression. The global repressor complex Tup1-Cyc8 was found to be the integrator of these two pathways, such that when nutrients are ample Tup1-Cyc8 binds to the IME1 promoter and represses IME1.

The mechanism by which the PKA and TORC1 signals are relayed to Tup1-Cyc8 is unknown. The central focus of my research is to understand how Tup1-Cyc8 integrates the nutrient signals at the IME1 promoter. I identified three transcription factors, Yap6, Sok2, and Phd1, that contribute to direct recruitment of Tup1-Cyc8 to the IME1 promoter. Binding of Tup1-Cyc8 is affected in cells without these transcription factors and IME1 is mis-expressed. Furthermore, these cells undergo meiotic divisions much earlier compared to control cells. Interestingly, in nutrient-rich conditions Tup1-Cyc8 is still bound to the IME1 promoter in these cells, suggesting that more factors are involved in maintaining Tup1-Cyc8 at the IME1 promoter. Taken together, more than three transcription factors are involved in integrating the nutrient signals that control entry into meiosis. I aim to dissect the mechanism of Tup1-Cyc8 recruitment at the IME1 promoter, and obtain a molecular understanding of how nutrient signals control entry into meiosis in budding yeast.

Keywords: *Meiosis, Nutrient sensing, Cell fate decision, Cell signalling, Gametogenesis, Sporulation, Cell cycle and cell division*

[PS4-25] Monitoring the Effect of Xylose on the Sugar Sensing Network in S. cerevisiae Through In Vivo Biosensors

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Saccharomyces cerevisiae is a promising platform organism for the development of lignocellulosic biorefineries due to its high fermentative capacity, industrial robustness, easy genetic manipulation and broad biotechnological applications. However, one of the main challenges with the utilization of lignocellulosic feedstock with Baker's yeast is the lack of functional assimilatory pathways for pentose sugars. Despite many successful metabolic engineering strategies to recombinantly enable xylose utilization in S. cerevisiae, xylose does still not seem to be recognized as a fermentable sugar by the yeast. This indicates that there might be possible issues with intra- and extracellular sensing of this sugar, which may, in turn, be of the main bottlenecks hindering an efficient xylose metabolism. We have recently reported on a panel of in vivo biosensors for single-cell, real-time monitoring of the sugar sensing of S. cerevisiae [1]. The sensors consist of a green fluorescent protein gene (yEGFP3) coupled with one of eight different endogenous yeast promoters known to be under control by at least one of the main glucose signaling pathways: Snf3p/Rgt2 (HXT1/2/4p), SNF1/Mig1p (SUC2p, CAT8p) and cAMP/PKA pathway (TPS1/2p, TEF4p). By comparing the signal during growth on different carbon sources, indications were found that extracellular xylose cannot be sensed by S. cerevisiae [1]. In order to better understand how xylose affects the sugar signaling pathways, we have expanded this study to also include strains capable of internalizing xylose. By increasing the understanding of the effect of xylose on the main sugar signaling pathways in S. cerevisiae, steps will be taken towards new metabolic engineering strategies to enhance xylose utilization.

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Keywords: *S. cerevisiae, sugar sensing, signaling, xylose, biosensors, flow cytometry*

[PS4-26] Lipids as Determinants of Drug Resistance in Candida albicans

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In both pathogenic as well non-pathogenic yeasts, several mechanisms can contribute to the development of multidrug resistance (MDR). Point mutations or overexpression of the drug

target, decrease in the import of drugs and an enhanced efflux of drugs are some of the strategies employed by drug resistant yeast to overcome the lethal effects of the drugs. However, extrusion of noxious compounds from the cell, mediated by efflux pumps is one of the most frequently used strategies for the development of drug resistance in yeasts, which holds true for several prokaryotic and eukaryotic organisms.

Pathogenic fungi C. albicans has also mastered several strategies to develop multidrug resistance (MDR) including over-expression of drug pumps. The two major efflux pump proteins involved in MDR in this pathogen belongs to ATP-binding cassette (ABC) and Major Facilitator (MFS) super families are both localized on the PM.

Recently, presence of the microdomains, also called 'Lipid rafts' in various organisms was found to play an important role in cell signalling, protein sorting, virulence and drug resistance. Lipid rafts are highly enriched in sphingolipid and ergosterol or cholesterol and are characterized by their insolubility in detergent. In yeasts also, we have previously shown that efflux pump proteins particularly of the ABC super family, are influenced by imbalances in membrane lipid composition. The presence of detergent resistant membranes (DRMs) within the yeast PM has recently been demonstrated. In order to critically evaluate the role of the DRM lipid constituents in the localization of the efflux pumps, we earlier overexpressed GFP tagged Cdr1p and CaMdr1p in different lipid mutant backgrounds of S. cerevisiae. The mutants were either defective in ergosterol or in the sphingolipid biosynthesis pathway. We demonstrate that the observed abrogated functioning of Cdr1p in the various mutant backgrounds is mainly due to its missorting because of which it remains poorly surface localized. CaMdr1p interestingly remains unaffected by the defects in the mutant strains.

In addition, there are reports which suggest the importance of phospholipids in drug resistance also. We checked the activity and translocation of these transporters in altered phospholipids background. To our surprise, even phospholipids affected the sensitivity of cells overexpressing these two drug transporters. However, their effect on translocation still needs to be verified.

Keywords: *candida, drug resistance, lipids, phospholipids, yeast, Rafts*

[PS4-27] Gdt1 and Erd1 Remove Byproducts of Glycosylation Reactions from the Golgi Complex and Alter Ca2+ Homeostasis

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Glycosylation reactions in the Golgi complex and the endoplasmic reticulum consume nucleotide sugars as substrates and produce inorganic phosphate (Pi) and acid (H+) as byproducts. Here we show that homologs of mammalian XPR1 and TMEM165 (termed Erd1 and Gdt1, respectively) recycle luminal Pi for reuse and exchange luminal H+ for cytoplasmic Ca2+, thereby promoting growth of yeast cells in low Pi and low Ca2+ environments. As predicted for reversible H+/Ca2+ exchangers, Gdt1 also promoted growth in high Ca2+ environments when the Golgi-localized V-ATPase was operational but had the

opposite effect when the V-ATPase was eliminated. Gdt1 activities were negatively regulated by calcineurin signaling and by Erd1, which recycled the Pi byproduct of glycosylation reactions and altered buffering of Ca2+ in the Golgi complex. Mutants lacking Erd1 exocytosed Pi into the environment and exhibited strong defects in Golgi glycosylation and Golgi to ER recycling of K(H)DEL proteins. Therefore, Erd1 can transport Pi in the opposite direction from humanb XPR1 and other EXS-family proteins. These findings shed new light on how the Golgi complex achieves its primary function while also contributing to H+, Ca2+, and Pi homeostasis.

Keywords: *Golgi complex, Calcium transport, Phosphate transport*

[PS4-28] Glucose and calcium-responsive phosphatases mediate crosstalk between organelle and plasma membrane proton pumps

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In S. cerevisiae, coordinated activity of the intracellular V-ATPase proton pump responsible for organelle acidification and the plasma membrane proton pump Pma1 is critical for pH homeostasis. Both chronic loss of V-ATPase activity in vma mutants and acute loss through treatment with V-ATPase inhibitors result in ubiquitination of Pma1 by the Rsp5 E3-ubiquitin ligase acting in combination with α-arrestin, Rim8. Approximately 50% of Pma1 is then internalized and degraded in the vacuole. Failure to internalize Pma1 when V-ATPase activity is lost causes very poor growth, indicating that endocytic downregulation of Pma1 is compensatory (Smardon and Kane (2014) J. Biol. Chem. 289:32316). The mechanisms for communicating loss of V-ATPase activity to plasma membrane Pma1 and designating 50% of Pma1 pumps for endocytosis are not understood. The negative genetic interaction between mutations required for Pma1 internalization and vma mutations allowed us to uncover additional candidates involved in this signaling pathway. Mutations in the Ca2+/calmodulin-dependent protein phosphatase calcineurin were previously shown to be synthetically lethal with vma mutants. We obtained vma2Δcnb1Δ mutants that grew very weakly at pH 5, and found that Pma1 is not internalized. Treatment of a vma2Δ mutant with calcineurin inhibitor FK506 resulted in inhibited growth, reduced Pma1 ubiquitination, and accumulation of Pma1 at the plasma membrane. The vma2Δ mutation was also synthetically lethal with the glc7-12ts mutation at semi-permissive temperatures and with a reg1Δ mutant, which lacks a regulatory subunit of the Glc7 (PP1) phosphatase. The vma2Δ glc7-12ts double mutant compromised Pma1 ubiquitination and retained Pma1 at the plasma membrane upon a shift to the non-permissive temperature. These data indicate that loss of V-ATPase activity is signaled to Pma1 through multiple pathways capable of sensing cytosolic Ca2+, glucose metabolism, and pH. We also asked whether simply reducing the level of cell surface Pma1 in a vma2Δ mutant would bypass the need for ubiquitination and endocytosis. However, a pma1-007 mutation, which reduces Pma1 levels by 50%, is synthetically lethal in combination with vma2Δ; this lethality was not suppressed by preventing Pma1 endocytosis with a rim8Δ mutation. This suggests that compensation for loss of V-ATPase activity may require populating endocytic compartments with Pma1, rather than simply reducing plasma membrane Pma1 activity.

Keywords: *proton transport, pH regulation, endocytosis, arrestin, calcineurin, vacuole, ubiquitination*

[PS4-29] Role of the ScMep2 Ammonium Transport Protein and Implication of its C-terminal Tail in Filamentation Induction

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Diploid Saccharomyces cerevisiae cells can switch from the yeast to a filamentous form of growth in conditions of nitrogen scarcity. This occurs for instance when a limiting ammonium concentration is provided as sole nitrogen supply (1). The transmembrane transport of ammonium is mediated by proteins of the conserved Mep-Amt-Rh family represented by three members in S. cerevisiae (Mep1-3) (2, 3). In several fungi, Mep2-type proteins are specifically required for filamentation in contrast to Mep1/3 orthologues and are proposed to act as ammonium sensors activating the dimorphic switch (4). However, the precise molecular mechanism of Mep2-mediated signal transduction remains unclear. We recently showed that the activity of Mep1/3 and Mep2 is controlled by TORC1 and its effector kinase Npr1 via distinct mechanisms (5, 6). Mep2 activity is fine-tuned by its C-terminal tail via the interplay between an enhancer domain activating substrate translocation via Mep2 hydrophobic core and an auto-inhibitory domain counteracting the action of the enhancer. Phospho-silencing of the auto-inhibitory domain occurs on S457 and involves a balance between the activity of TORC1-Npr1 and the redundant Psr1 and Psr2 plasma-membrane phosphatases. A Mep2S457D variant is active, and insensitive to TORC1-Npr1 control, with the C-terminal tail likely locked in a conformation preventing the auto-inhibitory domain to counteract the enhancer domain action. We combined the latter mutation with the D186N mutation, disrupting substrate recognition (7), to test whether a Mep2 variant unable to recognize ammonium but potentially locked in a putative constitutive active conformation could induce filamentation in the absence of substrate translocation. The Mep2D186N,S457D variant turned unable to transport ammonium nor to allow filamentation indicating that it is unable to signal constitutively. We currently address the role of Mep2 C-terminus in filamentation induction using specific variants bearing mutations/deletions in the auto-inhibitory and enhancer domains. We will present our first data sustaining a close link between ammonium transport efficiency of Mep2 and its capacity to allow filamentation.

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(2) Marini et al., Mol.Cell. Biol. 1997

(3) Marini et al.,Trends Biochem. Sci. 1997

(4) Lorenz et al., EMBO J. 1998

(5) Boeckstaens et al., Nat. Commun. 2014

(6) Boeckstaens et al., PLoS Genet. 2015

(7) Marini et al., Curr. Gen. 2006

Keywords: *Ammonium transport, Ammonium receptor, Filamentation signaling*

[PS4-30] Characterization of the Activity Regulation of Mep-Amt-Rh Ammonium Transport Proteins by TORC1-Npr1-Amu1/Par32 in Saccharomyces cerevisiae

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Ammonium is a major nitrogen source for microorganisms and plants, playing a critical role in the acid/base homeostasis in animals. In addition, ammonium is proposed to act as an inducer of autophagy in human tumor cells (1). Ammonium transport across cell membranes can be ensured by proteins of the Mep-Amt-Rh superfamily, conserved from bacteria to human, and represented by three members in S. cerevisiae (Mep1-3) (2, 3).

We have shown that the conserved TORC1 signaling pathway and its effector kinase Npr1 control the activity of all three Mep proteins via molecular mechanisms differing between the regulation of Mep1/3 and that of Mep2 (4, 5). The Mep2 activity is fine-tuned according to the quality of the nitrogen supply by phospho-silencing of a C-terminal auto-inhibitory domain, involving a balance between the activity of the Npr1 kinase and the redundant Psr1 and Psr2 plasma membrane phosphatases (4). The activity of Mep1 and Mep3 is in contrast controlled by a specific inhibitory partner, Amu1/Par32, a low complexity protein of unknown function regulated by TORC1-Npr1 (5). Under poor nitrogen supply, Npr1 promotes phosphorylation of Amu1 which appears mainly cytosolic while Mep1 and Mep3 are active. Upon preferred nitrogen supplementation, TORC1 upregulation enables Npr1 inhibition and dephosphorylation of Amu1 which accumulates at the cell surface and mediates the inhibition of Mep1 and Mep3. The latter mechanism of transport inhibition is reminiscent to the inhibition of prokaryotic ammonium transport proteins mediated by PII-type proteins, key nitrogen signal transducers widespread in bacteria and Archaea (6).

To characterize in molecular details the Mep1/3 regulation mediated by TORC1-Npr-Amu1, we performed a genetic screen and isolated hundreds of suppressors recovering Mep1 ammonium transport activity in the absence of Npr1 on minimal medium containing a low ammonium concentration as sole nitrogen source. We will present the structure-function analysis of the first mutations identified in Mep1 and in Amu1. Of note, a group of suppressors falls outside the MEP1 and AMU1 loci and is currently under investigation. This study might reveal new factors involved in the control of the transport activity of Mep proteins by TORC1.

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Keywords: *Ammonium, Transport, TORC1*

[PS4-31] New insight into mitochondrial retrograde signaling in the developing yeast colony

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During development, yeast colonies differentiate and form various cell subpopulations, which have different physiological and morphological characteristics. Previously, we have characterized two major cell subpopulations of U and L cells, which localize to upper and lower regions of smooth colonies of Saccharomyces cerevisiae strains, respectively. U cells with active TORC1 metabolically resemble tumor cells [1]. Recently, we have shown the important role of mitochondrial retrograde (RTG) signaling during colony development and cell differentiation [2]. The RTG pathway consists of Rtg1p, Rtg2p and Rtg3p activators, which mediate communication from mitochondria to the nucleus and activate expression of numerous genes. In our work we have shown that different branches of RTG signaling are active in different areas of the colony. We have identified new RTG pathway-regulated genes ATO1 and ATO2, which are expressed in U cells, being prominent marker proteins for these cells. Obtained data contribute to unraveling regulatory mechanisms involved in the formation and development of U cells. The project is supported by GAUK 95821, GAČR 15-08225S and SVV-2017-260426.

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[2] Podholová K., Plocek V., Rešetárová S., Kučerová H., Hlaváček O., Váchová L., Palková Z.: Divergent branches of mitochondrial signaling regulate specific genes and the viability of specialized cell types of differentiated yeast colonies; Oncotarget. 2016 Mar 29;7(13):15299-314.

Keywords: *Yeast colony development, Retrograde pathway, ATO genes, Mitochondria*

[PS4-32] Dynamic expression of Cit2p in developing yeast colonies is regulated by mitochondrial retrograde pathway

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Mitochondrial retrograde (RTG) pathway in yeast, known also as mitochondrial stress pathway in mammals, is conserved pathway that contributes to yeast cell survival under adverse conditions. S. cerevisiae has three key positive regulators of RTG pathway, Rtg1p, Rtg2p and Rtg3p, and three major negative regulators Mks1p, Bhm1p and Bmh2p. In our studies, we used colonies of S. cerevisiae as a model. We analysed colonies formed by strains deleted in genes for positive Rtg regulators as well as in genes for negative regulators of RTG pathway [1]. We found that dysfunction of RTG pathway decreases viability of specific subpopulation located in lower colony region. Using different strain constructs, we have found that RTG pathway is active in whole colony, but yet unknown co-regulators affect production of different target genes. CIT2 gene encoding citrate synthase, which is known marker of RTG pathway activity [2] has dynamic expression during colony development as shown by monitoring of Cit2p-GFP level. In acidic phase colonies Cit2p-GFP is produced in cells localized to upper colony regions, whereas its expression relocalizes to lower cells in colonies entering alkali developmental phase. Cit2p level is gradually decreased during colony ageing. Dysfunction of RTG pathway completely eliminates Cit2p production in colony cells. This project is supported by GAUK 958216, GAČR 15-08225S and SVV-2017-260426.

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Keywords: *Saccharomyces cerevisiae, retrograde pathway, mitochondria signaling, yeast colony, citratesynthase*

[PS4-33] Differences in metabolism within smooth and biofilm yeast colonies

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Yeast colonies have become an excellent model for studying various processes connected with microbial multicellularity, including differentiation into interacting cell subpopulations with specific features localized to particular positions within the structure. We have studied two Saccharomyces cerevisiae colony types: smooth colonies, formed by laboratory strains, composed of tightly packed cells and structured biofilm colonies, formed by wild strains. We have found that smooth colonies differentiate to at least two cell subpopulations: U cells and L cells that are specifically localized within the colony structure (Mol Cell 46:436, 2012; Oxidative Med. Cell. Longev. 2013:ID 102485, 2013; Cell Cycle 14: 3488, 2015). Unlike smooth colonies, part of the biofilm colony forms pseudohyphae

(”roots”) that invade the agar substrate. Also the surface (“aerial”) part of biofilm colony is composed of less tightly packed cells than cells within smooth colonies (J Cell Biol, 194: 679, 2011). Here, we compare metabolic/physiological and other properties of subpopulations forming smooth and structured colonies (submitted). Aerial and root cells exhibit some similarities to, but more differences from, U cells and L cells of smooth colonies. Whereas aerial cells of biofilm colonies behave like stationary-phase cells, upregulating catabolic pathways and stress-related genes, the U cells localized to upper parts of smooth colonies are metabolically active cells, activating many biosynthetic pathways, including protein synthesis, and have an active TORC1 pathway. In some of these properties U cells resemble more the subsurface pseudohyphae of structured colonies that are also metabolically active and activate many genes involved in ribosomal biogenesis and translation. Root cells thus differ from resting stressed L cells of smooth colonies. The numerous differences observed between aerial/U cells and root/L cells clearly reflect the different multicellular lifestyles of colonies and biofilms. This work was supported by GACR 13-08605S and 15-08225S and Norwegian financial mechanism 2009-14 No. 7F14083.

Keywords: *yeast colony and biofilm, metabolic differentiation, signaling and regulation*

[PS4-34] A Synthetic Feedback Loop Circuit as a Tool for the Study of the Yeast CWI Pathway

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The Saccharomyces cerevisiae cell wall integrity pathway (CWI) is responsible for the cell wall damage response necessary for the maintenance of this essential structure. Cell wall alterations, for example originated by chemical agents like Congo red, are detected by mechanosensors localized at the cell surface. Their stimulation lead to the sequential activation of a cascade of protein kinases operating in this pathway, including the MAPKKs Mkk1 and Mkk2 and the MAPK Slt2. Phosphorylation of Slt2 results in Rlm1 activation and the strong transcriptional induction of different genes, like MLP1.

In order to generate a tool for the identification of novel stimuli and regulatory components of this signaling route we have generated a genetic circuit named ‘IPAC’ (Integrity Pathway Activation Circuit) that promotes the hyperactivation of the pathway in the presence of a stimuli. To this end, the MKK1S386P allele, coding for a constitutively active allele of the CWI MAPKK, was placed under the control of the MLP1 promoter, which is strongly induced under stimulating conditions. Stimulation of the pathway in cells containing the IPAC activates this positive feedback loop resulting in cellular lethality. By screening a subcollection of deletion mutants we have identified several novel genes necessary for IPAC-mediated sensitivity to Congo red, zymolyase or SDS, suggesting that they are involved in the regulation of the CWI pathway. We have also identified novel compounds, such as neomycin sulfate, EDTA, LiCl or diphenhydramine hydrochloride that induce IPAC dependent lethality and Slt2 phosphorylation.

Keywords: *MAPK, Cell wall integrity, Feedback loop, Signaling pathway, Saccharomyces cerevisiae*

[PS4-35] The Condensation of the Metaphase rDNA by Stress is Dependent on TORC1 Complex

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The ribosomal DNA (rDNA) has served as a model to study chromosome condensation and segregation in the budding yeast Saccharomyces cerevisiae. Among the different structures seen under the microscope, the rDNA metaphase “loop” is a valuable tool to study the condensation process. How the rDNA condensation is affected by environmental stress is something that has remained poorly understood. We will show that the Heat-Shock (HS) can provoke the condensation of the rDNA. Since many of the roles in chromosome condensation and segregation have been established using temperature conditional alleles (ts), we have revisited the role of different factors using the auxin degron system (aid) to deplete protein function. With ts alleles, the rDNA loop structure is known to depend on the Condensin complex; whereas condensation and segregation require Cdc5 and Cdc14. We will show that we get to similar conclusions with the aid alleles. On the other hand, we further investigated the response of the rDNA chromosome structure under different stresses. Besides being affected by the HS, others stresses like calorie restriction, nitrogen starvation, and rapamycin treatment cause the compaction of the rDNA. We demonstrate here that this condensation process mediated by stress, is independent of Cdc14 and dependent on the TORC1 complex.

Grants that funded this work: BFU2015-63902- R (Ministerio de Economía y Competitividad) and PI12/00280 (Instituto de Salud Carlos III) to Félix Machín, both co-funded by EU-ERDF.

Keywords: *Heat-Shock, Rapamycin, TORC1, rDNA, Cdc14*

[PS5] Cell polarity, morphogenesis and cytoskeleton dynamics

[PS5-1] A New Factor Involved in the Establishment of the Non-random Inheritance Pattern of the Spindle Pole Bodies in Saccharomyces Cerevisiae

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Many cells, from yeast to higher eukaryotes, divide asymmetrically. During an asymmetric cell division, different polarized factors can segregate preferentially to only one of the two resulting cells, which acts as a mechanism for the generation of cellular diversity. An ideal model to study asymmetric division is the budding yeast Saccharomyces cerevisiae. Interestingly, in this organism the spindle pole bodies (SPBs, the equivalent of the centrosomes in budding yeast) are asymmetrically distributed during cell division, so that they display a non-random inheritance between the mother and bud. Specifically, the old SPB, which is inherited from the previous mitosis, is segregated to the daughter cell, while the new SPB, which is assembled de novo, is retained in the mother cell. Remarkably, asymmetric inheritance of the centrosomes has been also shown in higher eukaryotes, where the age of the centrosome can specify the fate of the cell and the disruption of this inheritance pattern can determine important consequences. We have identified a new factor that is necessary for the establishment of this non-random inheritance pattern of the SPBs between the mother and the daughter cell in budding yeast.

Keywords: *Mitosis, Asymmetric cell division, Spindle pole bodies*

[PS5-2] Actin Filament Initiation and Regulation at Cell Membranes

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Actin nucleation is the key rate limiting step in the process of actin polymerization, and tight regulation of this process is critical to ensure actin filaments form only at specific regions of the cell. Arp2/3 is a well-characterised protein complex that can promote nucleation of new filaments, though its activity requires additional nucleation promotion factors. The best recognized of these factors are the WASP family of proteins that contain binding motifs for both monomeric actin and for Arp2/3. Previously we demonstrated that the yeast WASP homologue, Las17, in addition to activating Arp2/3 can also nucleate actin filaments de novo, independently of Arp2/3. This activity is dependent on its polyproline rich region. Through biochemical analysis we have identified key motifs within the polyproline region that are required for nucleation and for elongation of actin filaments in the absence of Arp2/3. When expressed in cells, Las17 harbouring mutations in these

motifs, causes changes in the behaviour of endocytic reporter proteins prior to Arp2/3 recruitment, supporting a role early in actin filament initiation. Other experiments have allowed us to identify key phosphorylation sites involved in regulating actin binding, and regions of the protein conferring lipid binding properties. Overall our data support a model in which Las17 interacts with primed endocytic sites to directly generate and tether new actin filaments.

Keywords: *cytoskeleton, actin, actin-nucleation, WASP family proteins, membranes*

[PS5-3] The Pathway of Pulling Yeast Nuclei in Anaphase Controls Nuclear Motility at All Cell Cycle Stages in a Multinucleated Yeast

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Today, biologists want to understand the mechanisms of subsystems in cells. Biochemistry, for a long time extremely successful in developing amazing in vitro systems, can no longer handle mechanistic questions above a certain complexity. The only way to move forward is to use all experimental data gathered in the past to reconstruct the system in a mathematical model.

Our work demonstrates this approach by studying the coordination of movements of multiple nuclei in a filamentous yeast which evolved from budding yeast. Two pulling mechanism operate in S. cerevisiae, the Kar9-Bim1-Myo2 pathway for positioning of nuclei at the bud neck prior to nuclear division and the Dynein-Num1 pathway during pulling of nuclei through the bud neck. In the obligatory filamentous yeast Ashbya gossypii nuclei are highly motile performing bi-directional short- and long-range movements including nuclear by-passing. Homologs for all S. cerevisiae components involved in nuclear positioning and pulling are expressed in A. gossypii. However, experimental data accumulated over the past years had only identified the dynein motor, increased microtubule growth and shrinkage rates, budding yeast-like spindle pole bodies as microtubule organization centers, and Num1 (cortical dynein anchor) as key elements for the observed complex nuclear movements in A. gossypii.

This knowledge was implemented to run for the first time realistic simulations of movements of multiple nuclei in a common cytoplasm, which generated in vivo-like nuclear migration patterns. The simulations could also verify mutant phenotypes. We then exploited the simulation set up to study the influence of the number of microtubules per nucleus, an increase in cytoplasmic flow, and changes in organelle concentration on nuclear motility.

Keywords: *Evolution of nuclear dynamics, Microtubule dynamics, Dynein, Simulations*

[PS5-4] Specificity of cytosolic class II J protein, Caj1 in plasma membrane homeostasis in budding yeast

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Hsp40s, also known as J proteins are diverse group of molecular chaperones that partner with Hsp70s to perform an array of cellular functions. Via their highly conserved J domain, they stimulate the weak intrinsic ATPase activity of their partner Hsp70. Although the J domains are critical for J protein functions, the specificity of J proteins and the functional diversity of partner Hsp70s comes mainly from the ability of J proteins to either bind different client proteins or, tether Hsp70s to distinct sub-cellular compartments. Caj1 is one of the 13 J proteins that populate the cytosol of budding yeast. Caj1 is a non-essential class II J protein whose exact cellular role is still elusive. Here we used both deletion and over-expression approaches to investigate the role of Caj1 in S. cerevisiae. We show that cells lacking Caj1 show increased sensitivity to antifungal drugs and severe mislocalization of membrane proteins. On the other hand, over-expression of Caj1 was toxic and showed other pleiotropic effects such as temperature sensitivity, compromised plasma membrane integrity, pseudohyphal growth and cell cycle defects. Detailed analysis revealed that toxicity and perturbation of plasma membrane were C-terminal dependent and J domain independent. On contrary, appearance of pseudohyphal growth required both C-terminal as well as the J domain. Further, we identified complex genetic interactions between Caj1 and components of the ubiquitination machinery (UBI4), ergosterol biosynthesis pathway (ERG6), cell-cycle regulation (SWE1).The data presented suggests that Caj1 is involved in regulating plasma membrane homeostasis in budding yeast.

Keywords: *J protein, J domain, Caj1, Plasma membrane, Budding yeast*

[PS5-5] Role of Cdc42 Pathways in Regulating Group Cooperation and the Transition to Differentiated Multicellularity

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Group cooperation provides an evolutionary benefit and is thought to underlie evolutionary processes including the transition to multicellular life. The decision-making process surrounding group cooperation is poorly understood. Here, we identify decision-making pathways as key regulators of group cooperation. By exploring filamentous growth in unicellular fungal microorganisms, we describe a new response where cells assemble into multicellular aggregates to promote nutrient foraging. The Rho GTPase Cdc42, its effector MAPK pathway, and other signaling pathways regulated aggregate formation. Aggregates assembled by a mechanism where filaments from different groups interlocked to knit groups together, which resembled the intercalation of cells into tissues during mammalian development. This system was used to model the impacts of cheaters and handicapped individuals in aggregate assembly. Probing the limits of group cooperation showed that individuals adopt an optimal range of social interactions to balance foraging

needs. The uncanny resemblance between Cdc42 pathways in regulating group social responses in yeast and tissue development in metazoans led to the idea that decision-making pathways, by promoting group cooperation, may have contributed to the transition to multicellular life. This theory was supported by the roles GTPase pathways played in differentiated multicellular phenotypes in a laboratory selection experiment.

Keywords: *Rho GTPases, polarity, fungal pathogenesis, multicellular development, filamentous growth, signal transduction, Cdc42, MAPK*

[PS5-6] Spermine Modulates Yeast-Hypha Morphogenesis and Colony Morphology by an Acid Growth-Like Mechanism

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Polyamines play a regulatory role in eukaryotic cell growth and morphogenesis. Despite many molecular advances, the underlying mechanism of action remains unclear. Here, we investigate a mechanism by which spermine affects the morphogenesis of a dimorphic yeast of emerging relevance in biofuels biotechnology and plant interactions, Yarrowia lipolytica, through the recruitment of an auxin-dependent pathway involving an activation of the plasma membrane H+-ATPase. Morphological transition was followed microscopically and the plasma membrane H+-ATPase activity was analyzed in vitro and in vivo. Proton flux and acidification were directly probed at living cells surface by a non-invasive selective ion electrode technique. Spermine and indol-3-acetic acid induced the yeast-hypha transition, influencing the colony architecture. Spermine induced H+-ATPase activity and H+ efflux in living cells correlating with yeast-hypha dynamics. Pharmacological inhibition of spermine and auxin pathways prevented these physio-morphological responses, and indicated that it could act upstream of the auxin pathway. This study provides the first compelling evidence on the fungal morphogenesis and colony development as modulated by a spermine-induced acid growth mechanism analogous to those previously postulated for the multicellular growth regulation of plants.

Keywords: *Yarrowia lipolytica, polarized growth, acid growth theory, proton pump, scanning ion-selective electrode technique, polyamine*

[PS5-7] Analysis of the Role of PI4P in Prospore Membrane Extension during Sporulation of Budding Yeast

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Diploid cells of the budding yeast, Saccharomyces cerevisiae, form haploid spores in response to nutrient starvation. In this process, post-Golgi vesicles assemble at the

cytoplasmic surface of each spindle pole body and fuse homotypically forming de novo double membranes, termed prospore membranes (PSM). The PSM is distinctive in that it is derived from post-Golgi vesicles, which are redirected from plasma membrane (PM) to PSM, and that it dynamically changes its morphology, engulfing nucleus.

Previously, we identified spo73Δ as a mutant defective in PSM extension and spore formation. Spo73p contains a Dysferlin domain, an uncharacterized domain conserved in the mammalian proteins dysferlin and myoferlin. Though Spo73p is expressed specifically during sporulation and localizes to the PSM, the mechanism of PSM extension in which Spo73p is involved remains unclear. Through a genetic screen, we obtained two genes, a truncated allele of STT4 and the entire EFR3 gene, as multicopy suppressors of sporulation defect of spo73Δ. STT4 encodes phosphatidylinositol (PI) 4-kinase (PI4K), which forms a PI4K complex on the PM with two proteins, Efr3 and Ypp1.

In this study, we show that Stt4 localizes on the PSM where its product PI4P exists. In addition, through the analysis of multicopy suppressors, it is implicated that suppression of the sporulation defect of spo73Δ arose from dominant negative inhibition of Stt4 activity. To examine this possibility, we attempted to degrade Stt4 during sporulation specifically using an auxin-inducible degron (AID) system and found that degradation of Stt4 can partially restore PSM extension and sporulation of spo73Δ. Furthermore, selective depletion of PI4P on the PSM by a chimera of the Sac1 PI4P phosphatase domain fused to the PSM resident protein Dtr1, also suppressed the defect of spo73Δ. These results suggest that the decrease in PI4P levels on the PSM might contribute to extension of the PrM in spo73Δ.

To explore the downstream of PI4P, we compared the localization of markers, including membrane traffic marker proteins, structural proteins for membrane contact sites, or lipid transfer proteins, in wild-type and spo73Δ and found some candidates,. We are now verifying the relationship between these candidates and decrease in PI4P level on the PSM.

Keywords: *Sporulation, PI4P, Membrane morphology*

[PS5-8] Lipid-Dependent Regulation of Exocytosis in S. cerevisiae by OSBP Homologue (Osh) 4

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In many organisms polarized exocytosis is necessary for proper cell division or the functional specialization of cells. Knowledge of the mechanics of polarized exocytosis is therefore necessary to develop disease treatments or synthetic biology-based systems. In S. cerevisiae, polarized exocytosis is necessary for daughter cell formation and is dependent upon the OSH (Oxysterol Binding Protein (OSBP) Homologue) gene family, which encodes seven OSBP-related proteins (ORPs), any one of which is sufficient to support polarized cell growth. Family member Osh4p is sufficient, in the absence of other Osh family members, to support polarized exocytosis and functions late post-Golgi. In this study, in an oshΔ background, we used Osh4p a model to decipher ORP function in polarized exocytosis,

asking at what specific step in exocytosis does Osh4p function and whether PI4P or sterol binding by Osh4p is a requirement for this specific function. In vitro assays showed that Osh4p is necessary for the efficient docking, but not fusion, of exocytic vesicles at the plasma membrane (PM), in a PI4P-dependent manner. Osh4p defective in PI4P-binding also showed both significantly reduced co-localization with exocytic vesicles in vivo, relative to wild-type, as shown by fluorescence microscopy, and co-fractionation with Bgl2p, a marker of vesicles engaged in polarized exocytosis. This effect was membrane specific. Osh4p defective in either PI4P or sterol binding did not show decreased association with the PM. In contrast, lipid-free Osh4p associated with exocytic vesicles at wild-type levels but did not support vesicle docking at the PM, suggesting that the competitive binding of sterol and PI4P regulates the association of Osh4p with exocytic vesicles and is required for vesicle docking at the PM. Based on these data, we propose a two-step model for the regulation of polarized exocytosis, in which Osh4p first removes PI4P from exocytic vesicles and then exchanges bound PI4P for sterol at the PM to complete vesicle docking at sites of polarized growth. In addition, preliminary experiments with Osh6p, which binds phosphatidylserine rather than sterol, suggest that it is lipid exchange rather than lipid identity that regulates docking. Our study describes a specific in vivo role for lipid ligand binding by an ORP in an essential cellular process and guides our understanding of where and how ORPs may function in more complex organisms.

Keywords: *polarized exocytosis, oxysterol-binding proteins, OSBP-related proteins (ORPs)*

[PS5-9] Assembly of Septin Complex to Higher Order Structures

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Cytoskeletal proteins are involved in essential events of the cell division, process, in which the physical division of mother cell into two viable progenies has to be both spatially and temporally coordinated with the partition of replicated chromosomes. Septins are, together with the actomyosin contractile ring, core components, crucially involved in the cell division, notably in the cytokinesis. Septins are conserved guanosine phosphate–binding proteins present in most eukaryotic organisms. They serve at the division plane as a scaffold for other proteins and a diffusion barrier, which prevents movement of particular proteins between the mother and daughter cell. Despite of recent developments, it is still largely not understood at the molecular level how do the specific protein-protein interactions and posttranslational modifications control the septin organization, in particular via inducing changes in the polymerization of septin filaments and assembly of higher-order structures in vivo. Our ultimate goal is to understand the complex regulatory network of septins. Here, we have been depicting the role of two regulatory proteins of septins. First, Gic1 stabilizes septin filaments through bridging of pairs or multiple filaments and forming structures which resembles railways. We have been show, using yeast two-hybrid, deletion mutants and by decorating filaments with antibodies, that Gic1 binds specifically to septin Cdc10. Cdc42 in its active form binds to Gic1, which finally leads

to the dissociation of Gic1 from the septin filaments. Surprisingly, Cdc42-GDP in the absence of Gic1 directly interacts with septin filaments, resulting in their disassembly. Similar railways structures of septins have been also observed in vitro in the presence of Bni5, which crosslink adjacent septin filaments via interaction with Cdc11 subunits. We have also shown, that Gic1 and Bni5 bridge different septin subunits and stabilize septin higher-order structures at the bud-neck in distinct stages of the cell cycle. In addition, protein kinase Elm1 induces in the presence of Bni5 disassembly of long septin filaments, suggesting that these proteins may participate in the hourglass to double ring transition. In summary, interplay of small GTPases, accessory proteins and protein kinases was found to be crucial for the regulation of septin higher-order structures formation and dissociation during cell cycle.

Keywords: *cytoskeleton, septin, Gic1, Bni5, GTPase*

[PS6] DNA replication and cell cycle

[PS6-1] The adder phenomenon emerges from independent control of pre- and post-Start phases of the budding yeast cell cycle

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While it has long been clear that cells actively regulate their size, the molecular mechanisms underlying this regulation have remained poorly understood. In budding yeast, cell size primarily modulates the duration of the cell division cycle by determining the timing of the G1/S transition known as the Start. We have recently shown that cell growth dilutes the cell cycle inhibitor Whi5 in G1 to increase the rate of progression through Start. However, recent phenomenological studies in yeast and bacteria have shown that cells increase approximately a fixed volume during their entire cell cycle, independent of what size they are born. These results seem to be in conflict, as the phenomenological studies suggest that cells measure the amount they grow, rather than their size, and that size control acts over the whole cell cycle, rather than specifically in G1. Here, we propose an integrated model that unifies the adder phenomenology with the molecular mechanism of G1/S cell size control. We use single cell microscopy to parameterize a full cell cycle model based on independent control of pre- and post-Start cell cycle phases. We find that our model predicts the size-independent volume increase during the full cycle. This suggests that the adder phenomenon is an emerging property of several aspects of the budding yeast cell cycle rather than a causal consequence of an underlying molecular mechanism measuring a fixed volume increase.

Keywords: *size control, cell cycle, cell growth*

[PS6-2] An Assessment of Yeast Mitotic Catastrophe in Topoisomerase II Mutants: From Protective Factors to the Fate of the Progeny

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Topoisomerase II (Top2) is an essential protein due to its unique capability of removing catenations between the sister chromatids, a necessary condition for successful chromosome segregation in anaphase. For a long time, it has been known that most thermosensitive alleles of TOP2 (top2-ts) lead to a mitotic catastrophe which largely compromise survival of the progeny [1]. Nevertheless, what exactly happens to cells beyond anaphase is poorly understood. We will present a thoroughly study carried out with two broadly-used top2-ts, top2-4 and top2-5, and will show that the immediate progeny is largely impaired to continue the cell cycle. We will present data quantifying different contributors

to this deleterious phenotype and will show that a complex mixture of DNA damage checkpoints, programmed cell death and genetic imbalances explain the majority of cases. Besides, we have used hybrid diploids to assess what kind of genetic rearrangements are found in the few survivors. We will show that there is an unprecedented enrichment of Uniparental Disomies (UPD), in addition to many common gross chromosome rearrangements observed in other genome instability models. Finally, we will present the results of a genome-scale Synthetic Genetic Array (SGA) analysis where we looked for modifiers of top2-ts lethality. We will show that deficiency in the Mitotic Exit Network (MEN) greatly protects against top2-ts transient inactivation. Although this kind of protection is somehow expected and has been already shown before [2], the fact that it is the most important biological process picked up through SGA positions MEN as a putative coadjuvant target for Top2-related therapies.

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Keywords: *Top2, Mitotic Exit Network, DNA damage response, Cell Senescence, Cell death, Genetic instability*

[PS6-3] The evolution of the temporal program of genome replication

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DNA replication plays a major role in genome evolution by directly or indirectly promoting the formation of point mutations and large-scale chromosomal rearrangements. Conversely, both small and large-scale mutational events could possibly impact the evolution of the temporal program of genome replication itself. However, the evolutionary dynamics of the replication programs and the mechanisms by which the cohort of active replication origins is progressively renewed during evolution remain unclear. We experimentally determined the temporal program of genome replication in 10 related Lachancea species and characterized the evolution of their replication programs and origin usage at the genus level. We constructed families of orthologous replication origin to reconstruct the evolutionary scenario of origin gains and losses in the Lachancea phylogenetic tree since the species diverged from their last common ancestor. We inferred the functional properties of all replication origins, such as their respective chromosomal location, firing

time and efficiency, and revealed rules that govern the birth and death, or conservation, of active replication origin over evolutionary time.

Keywords: *Lachancea, Replication, Replication program, Replication origin, Genome, Evolution, comparative genomics, functional genomics*

[PS6-4] The RSC Complex Maintains Ploidy by Promoting Spindle Pole Body Duplication in Saccharomyces cerevisiae

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Ploidy, the number of complete sets of chromosomes an organism has, is tightly regulated in eukaryotic cells and is critical for cell function and survival. Cells must coordinate multiple pathways to ensure replicated DNA is segregated accurately to prevent changes in chromosome number. Here, we characterize an unanticipated role for the S. cerevisiae remodeling the structure of chromatin (RSC) complex in ploidy maintenance. We show that deletion of any one of six non-essential RSC genes causes a rapid transition from haploid to diploid DNA content due to non-disjunction events, resulting in increased fitness and in morphological changes. Furthermore, we find that RSC promotes spindle pole body (SPB) duplication by facilitating nuclear transport of the SPB component, Nbp1, through the nuclear pore complex (NPC). Once inside the nucleus, Nbp1 stabilizes Ndc1 at the SPB, which is a critical step for SPB insertion into the nuclear envelope. Thus, we provide novel insight into a role for RSC in NPC function, SPB duplication, and ploidy maintenance.

Keywords: *RSC complex, Spindle Pole Body, Ploidy Maintenance, Chromosome segregation*

[PS6-5] Whi7 and Whi5: a Tale of Two Players

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Start is the major control point in eukaryotic cell cycle. At this stage, cells decide to initiate or not a new round of cell division. Start involves the irreversible activation of a

transcriptional program by G1 CDK-cyclin complexes, being a key molecular event the inactivation of Start transcriptional repressors, Whi5 in yeast or Rb in mammals. In this work we demonstrate that Whi7 associates to G1/S gene promoters in late G1, acting as a repressor of SBF-dependent transcription. In fact, both Whi7 and Whi5 collaborate in Start repression binding simultaneously to promoters. Moreover, Whi7 is capable of substituting Whi5 function when is overexpressed, making of Whi7 a genuine paralog of Whi5. Interestingly, while Whi5 association to Start genes promoters completely depends on Swi6 and Swi4 (components of SBF), Whi7 binds mainly in a Swi4 dependent manner. This and other observations suggest that there could be a functional specialization between Whi7 and Whi5. Our results demonstrate that yeast cells, as occurs in mammalian cells, rely on multiple transcriptional repressors to block Start transition. Thus, understanding the interplay between Whi5 and Whi7 repressors could provide new clues about how distinct Rb family proteins interweave in mammalian cell cycle control.

Keywords: *Cell cycle, Start, Whi7, Whi5, G1/S transcription program, S. cerevisiae*

[PS6-6] Searching for New Genetic Factors and Environmental Conditions that Affect the Length of the Repetitive Ribosomal DNA Array in Saccharomyces cerevisiae

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The S. cerevisiae ribosomal DNA array (rDNA) has been widely used as model in significant genetic and cell biology problems, from chromosome organization and segregation to homeostasis of repetitive genomic sequences (Machín et al. 2005; Kobayashi 2011). The rDNA is comprised of about 100-200 copies arranged as head-to-tail tandem repeats of a basic 9.1 Kb unit that encodes precursors for all yeast ribosomal RNAs. The number of repeats seems to be exquisitely controlled by the cell and its homeostasis critically depends on Fob1 (Kobayashi 2011). Mutant strains for FOB1 lack the ability to steadily increase rDNA copy number when the rDNA array is significantively shortened. Nevertheless, both wild type and fob1Δ strains still suffer from sudden, and apparently spontaneous, big changes in the rDNA length. We have now used this phenotype to search for new environmental conditions and genetic factors that might influence this spontaneous rDNA “jumps”. Among the environmental conditions we will present data on stresses known to inhibit the master regulator TORC1 , which has been already shown to have a role in rDNA amplification (Jack et al. 2015). As for the genetic determinants, a complete screen of single mutants has been reported elsewhere (Kobayashi and Sasaki 2017), but now we have combined several double and triple mutations in a hypothesis-based approach that includes genes known to have roles in the rDNA stability and metabolism . Finally, we will present a model based in Fob1-aid (auxin-mediated degradation of Fob1) to further speed up the aforementioned studies.

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Keywords: *Fob1, rDNA, TORC1, copy-number change*

[PS6-7] A Novel Positive Role for BFA1, a Regulator of the Budding Yeast Mitotic Exit Network

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The mitotic exit network (MEN) is a Ras-like signaling pathway in S. cerevisiae that is critical for the proper execution of exit from mitosis, a result of B-type cyclin destruction. The key components of this pathway were identified two decades ago, but their regulation is still not well understood. The GTPase Tem1 functions at the top of the pathway to activate a series of kinases and ultimately, the phosphatase Cdc14. The localization of Tem1 to the spindle pole bodies (SPBs) is required for mitotic exit, but how the protein efficiently localizes to the SPBs is unclear. Here we show that overexpression of BFA1, a regulatory subunit of the two-component GAP complex that inactivates Tem1, blocks mitotic exit due to the mis-localization of Tem1 in these cells. This points to a novel positive function on the MEN for Bfa1 that is independent of its activity as a GAP complex. We further demonstrate that the overexpression of BFA1 does not impair the localization of the downstream MEN kinase Mob1/Dbf2. Lastly, we describe the identification of a suppressor of BFA1 overexpression that is likely a hyper-active allele of a MEN regulator. The budding yeast MEN components are homologous to regulators of the HIPPO tumor suppressor pathway and the oncogene centriolin. This suggests that our findings may lead to a better understanding of this important mammalian signaling pathway.

Keywords: *mitotic exit, MEN, Bfa1, Tem1*

[PS6-8] Bfa1 regulates microtubule dynamics to maintain mitotic arrest in response to misaligned spindle in S. cerevisiae

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The spindle position checkpoint (SPOC) of budding yeast delays mitotic exit in response to misaligned spindle for cell survival and maintenance of genomic stability. The GTPase-activating protein (GAP) complex Bfa1-Bub2, a key component of SPOC, inhibits GTPase Tem1 to induce mitotic arrest in response to DNA damage and spindle misorientation. However, our previous results showed that GAP activity is not necessary to block mitotic exit in response to misaligned spindle. The molecular mechanism of how Bfa1 controls mitotic exit to misaligned spindle remains to be elucidated. Meanwhile, other studies reported that regulation of microtubule dynamics is critical to maintaining mitotic arrest in response to mispositioned spindles. Here, we observed that the overexpression of Bfa1 increased the stability of astral microtubule (aMT) and interpolar microtubule independently of its GAP activity. These observations suggest that Bfa1 might modulate microtubule dynamics to control mitotic arrest in response to misaligned spindle independently of its GAP activity.

Keywords: *Spindle position, SPOC, Bfa1, microtubule*

[PS6-9] Gene Overexpression in Yeast Leads to Chromosome Instability by Impairing Cell Cycle Progression and Distorting the Redox Balance

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Hundreds of genes are involved in replication and partitioning of genetic material in the process of cell division. Genetic screens are used to find which of those genes and under what conditions are particularly important to stabilize the architecture of genomes. In mitotically dividing diploid cells, instability of chromosomes is manifested by frequent loss of heterozygosity (LOH). We used a complete collection of yeast strains with single genes cloned to a multicopy plasmid enabling abundant overexpression. We found that LOH was substantially enhanced, up to hundreds of times, for 39 overexpressed genes. As many as 32 of these genes were functionally related to cell cycle including chromosome segregation, cell cycle checkpoints and establishment and maintenance of cell polarity. Among them, only one coded for an enzyme directly involved in DNA metabolism. The remaining 7 genes were linked to metabolic processing of sulfur compounds. A likely outcome of overexpression of these genes was distortion of the cell's redox balance, increased activity of reactive oxygen species and damage of cellular compounds. More specifically, the metabolism of iron-sulfur clusters was possibly distorted and therefore enzymes which require these cofactors to function properly in the DNA metabolism were affected. One additional finding was that high transcriptional activity of 2-micron plasmids led to increased loss of whole chromosomes providing support to models linking segregation of such plasmids with mitotic segregation of chromosomes. In sum, our results show that strong overproduction of single proteins challenges the stability of genome in two ways: by impeding progression of necessary rearrangements in major cellular structures and by creating metabolic environment that is unfavorable for maintenance of macromolecules.

Keywords: *genome stability, chromosome instability, overexpression*

[PS6-10] Depletion of Yeast Topoisomerase II Activity Leads to the Formation of Recombination-Independent Branched DNA Structures at the Ribosomal DNA

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DNA replication is the process by which cells faithfully duplicate their genetic material prior to divide into two identical entities. To properly accomplish this goal, cells must to face several challenges, such as relaxing the positive supercoiling created ahead of the replication forks; an activity which requires either topoisomerase I or II (Top1 and Top2 in yeast) [1].

We will present a descriptive study about the progression through a synchronous cell cycle without Top2 activity by using the top2-5 thermosensitive allele. We will show that inactivation of Top2 does not stall the cell cycle, but instead cells go through mitosis and suffer a massive mitotic catastrophe. Strikingly, top2-5 cells modify their DNA such that it falls to enter a Pulsed-Field Gel Electrophoresis (PFGE), a behavior linked to the presence of branched structures such as DNA replication or recombination intermediates. We will demonstrate that this behavior is independent of executing cytokinesis and that it arises even before entering mitosis, specially at the ribosomal DNA locus (rDNA). Apparently, cells do not exhibit gross unreplicated chromosomes regarding to the isogenic control (TOP2), but they seem to create branched structures at the rDNA replication fork block, a condition which could be shared with other replication termination loci (known as fragile sites) [2]. We will also show by PFGE and neutral/neutral two-dimensional electrophoresis that depletion of classical homologous recombination proteins such as Rad52 does not reverse the appearance of DNA intermediates structures. These results point out the importance of Top2 at the time of finishing the replication process, which cannot be compensated by the classical homologous recombination mechanism.

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Keywords: *Top2, Branched Structures, Recombination, Replication, Cell Cycle, Genomic Instability*

[PS7] Mutagenesis, DNA damage and repair

[PS7-1] Physical Interactions Of Rad51 And Rad52 With Mcm2-7 Coordinate Their Binding To Chromatin During The Cell Cycle And In Response To DNA Damage

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The recombination proteins Rad51 and Rad52 help the fork to bypass blocking lesions and fill in the gaps of single-stranded DNA (ssDNA) generated during this process of DNA damage tolerance (DDT). In contrast to DNA double-strand breaks, Rad51 and Rad52 recruitment to the ssDNA lesions must occur during S phase. Here we show that Rad51 and Rad52 physically interact with the replicative helicase Mcm2-7 in G1. These interactions are lost during replication unless cells divide in the presence of replicative blocking lesions. They occur mostly in chromatin but are prevented at the pre-RC and at the replication forks, suggesting that Rad51 and Rad52 interact with the excess of Mcm2-7 helicases loaded in G1 and spread to the vicinity of the replication origins. Indeed, Mcm2-7 and Rad51 accumulate at a nuclease-insoluble chromatin fraction enriched in replication factors. Notably, these interactions coordinate the kinetics of chromatin binding of Mcm2-7, Rad51 and Rad52, which accumulate in G1, are released during S/G2 and are maintained in the presence of replicative DNA damage. This chromatin binding behavior is remarkable because homologous recombination is inactive in G1 and active during S/G2. Interestingly, the kinase activity of Cdc7 is required to preserve both the integrity of the Mcm2-7/Rad51/Rad52 complexes and the presence of these factors at chromatin during S/G2. Our results suggest novel roles for Cdc7 and Mcm2-7 in the regulation of the location of recombination proteins during DDT.

Keywords: *Replication, Homologous Recombination, Chromatin*

[PS7-2] Saccharomyces cerevisiae cells lacking SWI6 gene are genetically unstable

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Swi6 protein is known for its function in regulation of expression of genes necessary for DNA synthesis (G1/S phase of the cell cycle). It is also involved in the response to different stresses including cell wall stress, UPR stress and oxidative stress. We found that cells lacking SWI6 gene are oversensitive to DNA double strand breaks (DSB), are unable to properly repair DSB, and accumulate genome rearrangements. Genome instability of

haploid swi6Δ strains leads to high mortality of the cells or to premature senescence, while in diploid swi6Δ/swi6Δ strains aneuploidization occurs.

Comparative genomic hybridization experiments showed that in swi6Δ/swi6Δ strains chromosome V is duplicated. Two known swi6Δ suppressors, PAB1 and SWI4, are located on this chromosome. Overexpression of either of these genes increases the survival rate of cells lacking a functional copy of SWI6, whereas only Swi4, but not Pab1 is able to suppress the genome instability phenotype and influences the genome copy number. Studies employing newly identified suppressors of swi6Δ phenotypes indicate possible mechanisms leading to the genome instability in cells lacking SWI6.

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Keywords: *Saccharomyces cerevisiae, SWI6, SWI4, genome instability, double strand breaks, aneuploidy*

[PS7-3] Aging Yeast Preserves Acentric Circular DNA Elements

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Gene copy-number variations (CNVs) create much of the genetic variation that underlies genome evolution. Yet, detection of CNVs in a minute fraction of a cell population is a major challenge.

We reasoned that early processes in genomic rearrangements could be investigated by using an intermediate of CNV - extrachromosomal circular DNA (eccDNA). We previously demonstrated that eccDNAs are common in Saccharomyces cerevisiae, eccDNAs form from most parts of the yeast genome and eccDNAs can be purified with high sensitivity (Circle-Seq method, 1 eccDNA/2500 cells; Møller et al., 2015).

We now explored whether eccDNAs are maintained and enriched in aging yeast populations by measuring the eccDNA content in young and old S. cerevisiae cells, separated by 20-30 cell divisions.

We found that populations of aged yeast cells (generation ≥20) contained more than 50 % percent (53 - 70 %) of the eccDNA types also present in the parental populations (generation 0), suggesting that many eccDNAs are retained in aging yeast cells such as eccDNA from ribosomal RNA genes [rDNAcircles]. Maintained eccDNAs were more than 10 kilo-bases in average size, they contained several essential genes and carried core consensus sequences of replication origins. From 1e+6 diploid cells we detected 100 acentric eccDNAs on average and also an eccDNA with a centromere. Thus, the findings that acentric eccDNAs are common and that they can persist for more than 20 cell divisions suggests that they are important contributors of CNVs, both related to chromosomal deletions and amplifications.

Reference: Møller HD, Parsons L, Jørgensen TS, Botstein D, Regenberg B. Extrachromosomal circular DNA is common in yeast. Proc Natl Acad Sci. 2015 Jun 16;112(24):E3114-22. doi: 10.1073/pnas.1508825112.

Keywords: *Circular DNA, Copy number variation, Aging, Genome evolution*

[PS7-4] Combining the Mother Enrichment Program with High-Throughput Replica-Pinning to Identify Genes that Suppress the Accumulation of Mutations

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Defects in genes whose products act to maintain DNA integrity lead to genome instability, resulting in an overall increase of the spontaneous mutation rate. This condition, known as “mutator phenotype”, is thought to be a driving force during tumorigenesis.

An S. cerevisiae cell divides asymmetrically, yielding an (increasingly) ageing mother cell and a fully rejuvenated daughter cell. The Mother Enrichment Program (MEP) is an estradiol-inducible genetic system which allows daughter cell-specific inactivation of two essential genes, thus enabling the analysis of a cohort of ageing mother cells. When the MEP is active, daughter cells irreversibly arrest at the G2/M transition, while the mother cells keep dividing, resulting in a linear growth rate and the formation of microcolonies on agar medium. Occasionally, some cells become capable of evading the MEP due to acquired mutations: even in the presence of estradiol, these cells, called escapers, grow exponentially and form normal colonies on plates.

We developed a high-throughput replica-pinning approach to estimate spontaneous mutation rates in yeast strains, where estradiol-insensitive escaper colony formation serves as a readout for the occurrence of spontaneous mutations. With this protocol, we performed a genome-wide screen for genome maintenance genes. The MEP system was introduced in the yeast deletion collection using Synthetic Genetic Array technology. High-density arrays of MEP colonies were then grown for one week on agar plates in the presence of estradiol to allow for accumulation of spontaneous mutations in mother cells throughout their lifespan. Subsequently, colonies were further replicated on estradiol plates to detect the occurrence of escapers. High-throughput analysis of escaper formation frequency allowed identification of deletion mutants with elevated spontaneous mutation rate. Among many known mutator genes, we also found new genes involved in the maintenance of genome integrity.

Keywords: *Genome stability, Mutagenesis, Mother enrichment program, High-throughput screen*

[PS7-5] Recombination efficiency between subtelomeres is regulated by physical distance, DSB resection and chromatin compaction

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Genome integrity is insured by conserved DNA repair mechanisms among which Homologous Recombination (HR) uses an intact homologous sequence to repair a broken chromosome. Although the molecular steps of HR have been extensively described, their regulation in the context of the nucleus and in more or less compacted chromatin remains underexplored.

To understand how the different genomic, chromatin, and subnuclear contexts influence homologous recombination, we developed a new assay to score DSB induced recombination events between alleles located at different chromosomal positions and measure the competition between local gene conversion (GC) and break induced replication (BIR). We show that subtelomeric DSB are preferentially repaired by BIR despite the presence of consequent homology on both side of the break. Actively grouping telomeres, specifically increases GC rate between subtelomeres demonstrating that physical distance limits this process. GC between subtelomeres is also limited by Exo1 mediated resection and subsequent loss of the telomeric fragment suggesting that a race engages to find the recombination donor before loss of the homologous sequences surrounding the DSB. We show that heterochromatin spreading limits DSB resection in a process counteracted by EXO1 overexpression. We will discuss how this chromatin-dependent control could impact on the evolution of subtelomeric sequences and help to minimize the risks associated with repair events at chromosome ends.

Keywords: *DSB repair, Homologous recombination, Nuclear organisation, Chromatin*

[PS7-6] Phenotypic Expression of the Primary DNA Lesions Through the Cell Cycle in Yeast Saccharomyces Cerevisiae

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Primary DNA lesions induced by endogenous or exogenous genotoxic agents alter the structure of DNA, affect replication and transcription and, as a result, negatively influence the transfer and realization of genetic information. We have investigated the ability of primary DNA lesions to pass through the cell cycle stages. To study processing and phenotypic expression of primary lesions during the cell cycle we used the alpha-test that is based on genetic system controlling mating types in heterothallic yeast Saccharomyces cerevisiae. The alpha-test allows to detect phenotypic expression of primary lesions before they are eliminated by repair or converted into gene mutations, recombination, gene conversion, chromosome and chromosome arm loss. Using the alpha test, we determined

the frequency of inherited and non-inherited genetic changes induced by UV radiation in asynchronous yeast cultures and in mutant strain cdc28-4 blocked at the G1 stage. Our results have shown that phenotypic expression of primary DNA lesions in the alpha-test depends on the type of primary lesions and the stage of the cell cycle in which this lesion occurred. Primary lesions that occurred in the G1 stage of the cell cycle can immediately lead to a mating type switching in yeast cell from alpha to a, and express phenotypically on the same stage of the cell cycle. Phenotypic expression of primary lesions, occurring in the S or G2 stages of the cell cycle, in the alpha-test depends on their ability to break replication. We have shown that heterothallic yeast cells do not need to undergo a complete cell cycle to get rid of products specific for the α-mating type cells, and they can mate at the same G1 stage, at which this switching of the mating type is occurred. Additionally, we found the loss of chromosome III does not lead to the instant death of yeast cells, induces a mating type switching from alpha to a and does not disrupt their ability to hybridize. Supported by grant of St. Petersburg State University 1.38.426.2015, and the Russian Science Foundation project 14-50-00069.

Keywords: *Primary DNA lesions, alpha-test, mutagenesis, cell cycle*

[PS7-7] Molecular characterization of R-loops causing genetic instability in S cerevisiae

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R-loops, structures formed by a RNA-DNA hybrid and a displaced single-stranded DNA molecule, are naturally involved in many different cellular processes, such as the replication of E. coli plasmids or mitochondrial DNA, as well as in class-switch recombination of immunoglobulin genes in B-cells. In certain abnormal circumstances, the co-transcriptional formation of R-loops can lead to replication fork impairment with deleterious consequences for genome integrity. To date, such aberrant DNA-RNA hybrids have been detected through a battery of both indirect and direct tools. However, at present, neither their length or the frequency nor whether these hybrids are continuous or discontinuous is known. With the aim of further characterizing R-loops, we have mapped and counted the DNA molecules containing DNA-RNA hybrids at the molecular level by the bisulfite modification assay. We will present the results of the length of R-loops and the frequency at which they are formed in several mutant strains of S. cerevisiae, containing mutations in genes involved in different processes such as mRNA processing or chromatin remodelling. The implications for the role of R-loops in transcription-associated genetic instability will be discussed.

Keywords: *THO mutants, R-loops, bisulfite, genomic instability, H3S10P*

[PS7-8] Hpr1 and Sen1 conditional mutations provide new insights into R loop-mediated genome instability

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R loops are RNA:DNA hybrids naturally formed during cellular processes that can also compromise genomic integrity when occurring out of their natural context. Cells have developed mechanisms to prevent or eliminate harmful R loops. Two well-studied proteins involved in R loop homeostasis and genome stability maintenance are Hpr1 (hTHOC1), which functions in mRNPs biogenesis, and Sen1 (hSETX), an RNA/DNA helicase that participates in transcription termination. Our laboratory has shown that R loops are linked to histone H3 S10 phosphorylation (H3S10-P), a mark of chromatin condensation, consistent with a role of RNA in chromatin structure. Aimed at identifying the molecular basis of the connection of RNA-DNA hybrids with DNA damage, chromatin changes and replication defects after prompt Hpr1 or Sen1 depletion, we have generated Saccharomyces cerevisiae hpr1- and sen1-degron strains in which proteins can be degraded in a controlled manner. In contrast to previously published analyses, this approach avoids cell adaptation that could mask some phenotypes in hpr1Δ or sen1-1 strains, and permits the analysis of the cells after immediate Hpr1 or Sen1 depletion. Our results show that depletion of either protein causes genetic instability (high levels of recombination frequency and Rad52 foci) and a significant delay in replication fork progression, as previously observed in hpr1Δ and sen1-1 mutants. Interestingly, hpr1-degron cells present R-loop accumulation and new chromatin marks in specific DNA regions that were not previously observed in null hpr1Δ cells. These data will be discussed in the context of R loop-induced genome instability in association with chromatin modifications.

Keywords: *R loops, genomic instability, hybrids, chromatin, degron, THO mutants, depletion, H3S10-P*

[PS7-9] Decoding the Role of PKC in DNA Integrity Checkpoint

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The protein kinase C (PKC) superfamily plays important regulatory roles in numerous cellular processes. Saccharomyces cerevisiae contains a single PKC, Pkc1, whose main function is cell wall integrity maintenance. Recently, we have described that Pkc1 and the mammalian isoform PKCδ control DNA integrity checkpoint activation, indicating that this mechanism is conserved from yeast to humans. In this work, we carried out different approaches in order to better understand the role of PKC in response to genotoxic stresses.

Our previous results indicated that Pkc1 acts in the early stages of the DNA damage-signalling pathway. We analysed the recruitment of Ddc1 (subunit of the 9-1-1 checkpoint clamp complex) and Ddc2 (protein interacting with the upstream checkpoint kinase Mec1). After the induction of a DSB there was no activation of the checkpoint in a pkc1 mutant strain. Nevertheless, both Ddc1 and Ddc2 associate to the DNA damage site. A physical interaction between PKCδ and the mammal ortholog of Ddc1 has been described in DNA damage conditions. However, neither physical nor genetic interactions between Pkc1 and Ddc1 have been detected in yeast. Looking for the minimum PKC fragment involved in the

DNA damage response, we have observed that truncated versions of Pkc1 lacking different regulatory domains are still able to activate the checkpoint pathway. In the case of PKCδ, a unique activation mechanism absent in the other PKC isoforms has been described. This mechanism involves the A-helix (a key motif only present in PKCδ), activation loop and C-helix. Mutations in some residues of these components suggest that this mechanism is crucial for PKCδ ability to activate the DNA damage response in yeast. Finally, we found in yeast that Pkc1 and PKCδ delocalize from sites of polarised growth in response to DNA damage. Our research will provide a better understanding of the Pkc1 and PKCδ role as key players in the DNA checkpoint pathway. Moreover, it will clarify the similarities and differences in their regulatory function in the activation of the checkpoint pathway.

Keywords: *DNA damage, Checkpoint, Pkc1, PKCδ, S. cerevisiae*

[PS7-10] Two Faces of Control of DNA Polymerase ζ in Yeast Saccharomyces cerevisiae by Fe-S Cluster Associated with the C-Terminus of the DNA Polymerase δ

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Eukaryotes have about 15 different DNA polymerases (pols) whose activity and fidelity broadly varies. Most of the genomic DNA in eukaryotes is synthesized by the accurate processive replicative polymerases polα, polδ and polε, with some involvement of a specialized error-prone polζ, which is recruited when DNA synthesis is impeded by DNA lesions, imbalanced dNTP pools or non-B DNA structures. Attraction of the polζ for replication prevents major genome rearrangements and cell death in expense of the increase of rates of point mutations. Strict control of the proper involvement of each DNA pol during replication or repair is essential for genome stability. Loss of these controls leads to increased mutation rates, changes in the evolutionary fate of cells and leads to diseases, including cancer. Several molecular mechanisms controlling pols switches during replication and repair have been described. None of them are fully understood. We focus on the mechanism by which C-terminal domains of the B-family polymerases control the polymerase switch during replication. DNA polymerases polδ and polζ have conservative cysteine rich motifs in the C-terminus (CTD), which are required for binding Fe-S clusters. We propose that Fe-S clusters are essential for the communication between DNA polymerases and regulate their switches during replication on damaged DNA. We constructed yeast S. cerevisiae strains with a change of the conservative cysteine in CTD of the polδ (pol3-13 allele). We have confirmed that the pol3-13 mutant is UV-sensitive and is defective in UV light induced mutagenesis. At the same time, the pol3-13 mutation leads to the five-fold increase of spontaneous mutation rates. This effect was completely depended on the functional polζ. We propose that Fe-S cluster in the catalytic subunit of polδ plays an essential role in UV mutagenesis, in either a step preceding or following the action of polζ. At the same time, this function of the Fe-S cluster is not required for the generation of spontaneous polζ-dependent mutations under replication stress. Our results reveal the

differential control of spontaneous and UV induced polζ-dependent mutagenesis by the Fe-S clusters present in replicative polδ.

Keywords: *Replication, DNA polymerases, Genome instability, Fe-S cluster*

[PS7-11] Irc5 DNA Translocase Promotes Cohesin Association with Chromatin

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Cohesin is a multiprotein complex that holds sister chromatids together until the onset of anaphase ensuring correct sister chromatid separation during mitosis and meiosis. In addition to its role in chromosome segregation, cohesin was shown to play important cohesion-independent functions. These include regulation of repetitive regions like rDNA and DNA repair at both stalled replication forks or DNA double-strand breaks. Here we report that Irc5, a member of the Swi2/Snf2 ATPase family, is a novel interactor of cohesin and promotes cohesin binding to chromosomes by providing optimal chromatin environment for cohesin loading. We show that disruption of IRC5 or its translocase activity leads to reduced levels of cohesin at centromeres and chromosome arms resulting in mild precocious sister chromatid separation. Moreover, cells lacking IRC5 had less cohesin associated with rDNA leading to increased levels of unequal recombination between rDNA repeats resulting rDNA repeat loss. Finally, we show that under replication stress conditions Irc5 promotes cohesin accumulation at stalled replication forks and replication resumption. Deletion of IRC5 results in DNA damage accumulation that causes delay in replication completion leading to increased sensitivity to MMS.

Keywords: *Irc5, cohesin, cohesin loading, chromatin remodeling, replication stress*

[PS7-12] Position or Chromatin Structure: Causal Effects on Double-Strand Break Repair Pathway Choice

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Double strand breaks (DSBs) are the most dangerous form of DNA damage for cell survival. They can occur naturally by replication fork collapse or artificially induced by genotoxic agents. Repair of DSBs can be performed by different modes, Homologous Recombination (HR) / Non Homologous End Joining (NHEJ). DSB has consequences on chromatin both at the local and global by changing chromatin properties, such as stiffening (Herbert et al., EMBO 2017) which are instrumental to the DNA damage response (DDR). In addition, it is now clear that the non-random organization of chromosomes hase a key role in DNA metabolism. For instance, in the presence of sequence homologous to the damaged region, nuclear organization has a key role in the repair of DSBs by HR (Agmon et al., 2013).

However, the specific role of chromosome organization on chromatin structure towards DSBs repair remains to be understood.

To ask for the specific role of spatial or genomic position in the DSBs repair mode, we develop here a system using natural cutting site based on the use of a restriction enzyme (AsiSI) previously established in mammals (DIVA, Iacovoni et al., 2010). By the presence of 38 natural cutting sites recognized by the AsiSI endonuclease, we can study the response to multiple DSBs while knowing the spatial and genomic position where DSBs occurred.

We show that AsiSI is transcribed, translated, induces a DDR checkpoint response and the formation of Rad52-GFP foci (Lisby et al., 2001). Furthermore, and surprisingly, an effect on survival is significant in Rad52 strain but not in a Ku70, indicating the prominent role of HR in AsiSI DSBs repair.

As DSB are known to induce chromosome mobility, we have analyzed chromosome motion upon AsiSI DSB. We show an increase in mobility after AsiSI induction for a peripheral site and, conversely, no modification for a luminal site. Perhaps as a consequence, the interactions between peripheral domains are more frequent after AsiSI induction. Moreover, the populational analysis by Q-PCR shows a differential DSBs level at each site according to the predicted position in the nuclear space.

Altogether, our result indicates a preferential repair pathway linked to spatial organization.

Keywords: *Chomosome organization, DNA damage, DNA repair*

[PS7-13] A genome wide analysis of disconnect between binding and effect of chromatin factors

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Chromatin factors can modify the chromatin states by chemical modifications and nucleosome remodeling. More than 400 such proteins are known in yeast and genome wide location analysis of a large number of chromatin factors shows a wide spread binding within the promoter regions of gene, but often lacks effect on transcription levels of bound genes when these factors are knocked down, a phenomena known as disconnect between binding and effect. In this study, using publically available genome-wide datasets, we suggest that functional buffering and condition specific transcriptional roles of chromatin factors did not account for the observed disconnect. Instead the expression levels of both chromatin factors as well as their bound target genes were cell cycle specific, with factors showing no effect on transcription being associated with G1/S phase and factors showing effect on transcription being associated with G2/M phase transcription of metabolic genes. Further, the gene ontology analysis of protein interaction partners of these chromatin factors showed significant enrichment of DNA replication and DNA repair related activities for the chromatin factors which exhibited disconnect, indicating their potent role in

maintaining the genome integrity in G1 and S phase. The unaffected bound genes had fewer single nucleotide mutations, DNA breaks and cryptic transcripts compared to affected genes, further highlighting the significant role of chromatin factors in guarding the promoters against genetic and epigenetic errors. We validated our results for candidate chromatin factors by using yeast genetics approaches. When challenged with mutagenic agent such as HU or MMS, we observed significantly higher frequency of mutations at bound promoters of chromatin factors exhibiting disconnect in the mutant strains of that factor as compared to wild type strains. Accordingly, the knock out strains of chromatin factors exhibiting disconnect showed greater fitness defect when grown across different environmental conditions. Altogether, our results suggested that wide spread binding of chromatin factors might stem from their role in guarding the DNA promoters from possible damage and their causal role in transcription should be dealt with caution.

Keywords: *Chromatin Factors, DNA Repair, Mutagenesis*

[PS7-14] Role of CK2 in S. cerevisiae Ty1 LTR-retrotransposon Replication Cycle

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Ty1, the most abundant and active Long Terminal Repeat (LTR)-retrotransposon in S. cerevisiae, preferentially integrates within a 1-kb window upstream of RNA Polymerase III (Pol III)-transcribed genes. The integration step is performed by Ty1-encoded integrase (IN). Recently, we identified an interaction between Ty1 IN and the Pol III subunit AC40 and demonstrated that AC40 is the predominant determinant of Ty1 integration upstream of Pol III-transcribed genes. Loss of AC40/IN interaction dramatically alters Ty1 integration profile, leading to a redistribution of Ty1 insertions in the genome, mainly to chromosome ends.

To better understand the molecular bases of Ty1 integration site selection, we have performed proteomic approaches to identify IN cofactors in vivo. By these approaches, we have confirmed the interaction of IN with Pol III. We have also identified the four subunits of the Ser/Thr casein kinase 2 (CK2) and confirmed the interaction between IN and CK2 by co-immunoprecipitation. Since CK2 controls Pol III transcription, it might also control IN/Pol III interaction or Ty1 integration at tRNA genes. We found that individually, the absence of each CK2 subunit did not affect Ty1 integration upstream of Pol III-transcribed genes, neither Ty1 overall integration frequency. However, the concomitant absence of a regulatory and a catalytic subunit of CK2 drastically increased Ty1 overall integration frequency and targeting upstream of Pol III-transcribed genes.

Keywords: *retrotransposons, Ty1, CK2*

[PS7-15] Chromosomal association of the SMC5-6 complex is dependent on interaction of its Nse1-Nse3-Nse4 subcomplex with DNA

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SMC5/6 is a highly conserved protein complex related to cohesin and condensin, which are the key components of higher-order chromatin structures. The SMC5-6 complex is essential for proliferation in yeast and is involved in the homologous recombination-based DNA repair processes, including repair of DNA double strand breaks, in replication forks stability and processing, and in cohesin dynamics regulation. However, the precise mechanism of action of SMC5-6 is not known. We will present evidence that the NSE1/NSE3/NSE4 sub-complex of SMC5-6 preferentially binds to double-stranded DNA over single-stranded DNA. Mutations of key basic residues within the NSE1/NSE3/NSE4 DNA-binding surface reduce binding to DNA in vitro. Their introduction into the Schizosaccharomyces pombe genome results in cell death, or chromatin aberrations and hypersensitivity to DNA damaging agents. Chromatin immunoprecipitation analysis of the hypomorphic nse3 DNA-binding mutant shows a reduced association of fission yeast SMC5-6 with chromatin. Based on our results, we propose a model for loading of the SMC5-6 complex onto the chromatin.

Keywords: *structure maintenance of chromsome, SMC5/6 complex subunits, DNA repair/replication*

[PS8] Regulation of transcription, splicing, mRNA export and stability

[PS8-1] Coordination of Stress-Mediated Gene Expression and DNA Replication by Signaling Kinases

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Exposure of cells to osmostress results in the activation of the Hog1 stress-activated protein kinase (SAPK). Activation of this highly conserved MAP kinase is required to generate a set of osmoadaptive responses essential for cell survival. Adaptation to osmostress requires the induction of a large number of genes as well as the control of cell cycle progression. Upon stress, there is a major down-regulation of gene expression that is bypassed specifically in stress-responsive genes by the action of the Hog1 SAPK which acts in multiple steps of mRNA biogenesis including transcription initiation and elongation (Nadal-Ribelles et al., 2012; Nadal-Ribelles et al., 2014; Nadal-Ribelles et al., 2015). In addition to regulate transcription, SAPKs control cell cycle progression. During S phase, the Hog1 SAPK is critical to coordinate transcription and replication by directly phosphorylating Mrc1, allowing for full stress-responsive transcription without affecting DNA integrity (Duch et al., 2013). Remarkably, in addition to osmostress, other stresses also induce a large number of genes during S phase. These transcriptional outbursts cause genomic instability and transcription-associated recombination in cells carrying a non-phosphorylatable Mrc1 mutant. By a systematic biochemical assay we have identified several kinases that are able to phosphorylate Mrc1 in the same phosphorylation sites than Hog1. This indicates that Mrc1 can integrate signals from multiple kinases to delay replication when an outburst of transcription occurs during S phase. All together highlights the relevance of the signaling kinases in the control of gene expression and cell cycle regulation.

Keywords: *SAPK signaling, cellular stress, gene transcription and cell cycle regulation*

[PS8-2] Dal80 GATA factor intragenic occupancy is independent of its target sequences in the ORF and correlates with high mRNA abundance in budding yeast

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Yeast nitrogen catabolite repression (NCR) is a paradigm for metabolic adaptations to environmental changes. Key factors involved in this process are four GATA transcription factors (Gln3, Gat1, Dal80 and Gzf3), which are active in conditions of nitrogen limitation (proline), bind upstream of NCR-sensitive genes and regulate their expression. Although the consensus binding site for these factors has been characterized a while ago and is dispersed throughout the genome, the precise set of regulated genes was still lacking. We performed a genome-wide analysis of Dal80 binding (ChIP-Seq), correlated our results with GATA site occurrence and with GATA factor-regulated gene expression (RNA-Seq) in proline-grown cells. ChIP-Seq analyses revealed that Dal80 binds to ORFs in addition to gene promoters and this binding correlates with high gene expression. ORF binding was confirmed at a large set of characterized NCR-sensitive genes, and we showed that this occurred as a consequence of elongation, independently of GATA sequences that may be present in the ORF.

This work, GI and ED is funded by the Commission Communautaire Française (COCOF). AR was a FRIA research fellow.

Keywords: *Transcription, ChIP-Seq, NCR*

[PS8-3] The CCAAT-Binding Complex Controls Respiratory Gene Expression And Iron Homeostasis In Candida glabrata

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Candida glabrata is a fungal human pathogen close to the model yeast S. cerevisiae. Candida glabrata is accounting for more and more systemic candidemia in immunodepressed patients, with a high mortality rate. Compared to Candida albicans, the most prevalent pathogenic yeast, little is known about the transcription factors that control Candida glabrata's adaptation to varying environments and allow it to survive and thrive in the human body. In the frame of the CANDIHUB ANR project, we are using genome-wide chromatin immunoprecipitation analyses (ChIP-seq) and transcriptomics on mutants for particular transcription factors to achieve a large scale description of the transcriptional regulatory networks associated with stress response in this species. This poster is especially focused on the results we obtained for the role of the CCAAT-binding complex (CBC) in the control of respiratory genes expression and iron homeostasis in Candida glabrata. The CBC is a heterotrimeric transcription factor which is widely conserved in eukaryotes. In the model yeast S. cerevisiae, CBC positively controls the expression of respiratory pathway genes. This role involves interaction with the regulatory subunit Hap4. In many pathogenic fungi, CBC interacts with the HapX regulatory subunit to control iron homeostasis. HapX is a bZIP protein which has in common with Hap4 only the Hap4Like domain (Hap4L) required for its interaction with CBC. Here, we show that CBC has a dual role in the pathogenic yeast C. glabrata. It is required, along with Hap4, for the constitutive expression of respiratory genes and it is also essential for the iron stress response, which is mediated by the Yap5 bZIP transcription factor. Interestingly, Yap5 contains a vestigial Hap4L domain.

The mutagenesis of this domain severely reduced Yap5 binding to its targets and compromised its interaction with Hap5. Hence, Yap5, similarly to HapX in other species, acts as a CBC regulatory subunit in the regulation of iron stress response. This work reveals new aspects of iron homeostasis in C. glabrata and of the evolution of the role of CBC and Hap4L-bZIP proteins in this process.

Keywords: *Stress response, Regulation of gene expression, Network, Iron homeostasis*

[PS8-4] How Yap8 couples arsenic-sensing to transcriptional regulation of target genes

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The transcription factor Yap8 is critical for arsenic tolerance in the yeast Saccharomyces cerevisiae [1]. Yap8 is a member of the evolutionary conserved AP-1 family of bZIP transcription factors, and is the first arsenic sensor protein described in eukaryotes [2]. Currently, little is known about the molecular mechanisms by which Yap8 couples arsenic-sensing to transcriptional regulation of target genes. Our data are consistent with a model in which a DNA-bound form of Yap8 acts directly as an arsenite [As(III)] sensor, and that As(III) binding to Yap8 acts as a molecular switch that converts inactive Yap8 into an active transcriptional regulator [2]. Preliminary data indicates that Yap8 triggers chromatin remodelling to efficiently induce gene expression: nucleosome scanning, chromatin immunoprecipitation and gene expression assays show that histone occupancy decreases whilst RNA polymerase II occupancy and target gene expression increase in an As(III)- and Yap8-dependent manner. Taken together, our work has shed novel light on Yap8 function and advanced our molecular understanding of one of the largest families of transcription factors in eukaryotic cells.

Keywords: *arsenite, metalloid, arsenic sensing, Yap8, transcription factor, gene expression, AP-1 family, yeast*

[PS8-5] P-bodies regulate transcriptional rewiring during DNA 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 degradation and/or storage of mRNAs. We recently discovered that P-bodies form in yeast in response to DNA replication stress induced by HU (hydroxyurea). 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. Here, we aimed to identify mRNAs that are processed by P-bodies during replication stress. First, we performed a transcriptome study on lsm1Δ cells upon acute HU exposure to identify mRNAs that are stabilized in the absence of a

functional P-body-dependent mRNA degradation pathway. Second, we used an SGA-based suppressor screen to identify genes whose expression is toxic in the absence of Lsm1 and Pat1 during replication stress. We found that the transcriptome in lsm1Δ is altered both during normal growth and during replication stress, with more than 800 mRNAs being stabilized in lsm1Δ compared to wild type. Interestingly, we found that inactivation of the coding sequence of 6 of those 800 mRNAs was able to suppress HU sensitivity of lsm1Δ and pat1Δ strains suggesting that these genes encode mRNAs that need to be degraded in a P-body dependent manner upon HU exposure. Among these, we identified YOX1, a gene encoding a transcription repressor critical for the regulation of cell cycle and DNA replication genes. Consistent with P-bodies regulating YOX1 mRNA abundance, we found that YOX1 mRNA localizes to P-bodies and accumulates at P-bodies in the absence of the mRNA exonuclease Xrn1. To gain insight into the role of Yox1 during replication stress, we identified 156 genes that are down-regulated upon YOX1 overexpression. Among this set of targets, we found that de-repression of ALD6, encoding a cytoplasmic acetaldehyde dehydrogenase, is critical for replication stress resistance. Indeed, accumulation of acetaldehyde, the substrate of Ald6, is strongly toxic for cells experiencing DNA replication stress. Together, our data suggest a model where YOX1 mRNA abundance is post-transcriptionally regulated by P-bodies in order to reduce the level of the Yox1 transcription repressor and therefore prevent repression of genes necessary for survival of DNA replication stress.

Keywords: *P-bodies, RNA decay, DNA replication stress, Acetaldehyde*

[PS8-6] Function and Factors of mRNA Stability Changes Upon a Nitrogen Upshift

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Changes in mRNA stability can hasten the reprogramming of the yeast transcriptome to adapt to sudden environmental changes. We measured the changes in mRNA degradation rates of the whole yeast transcriptome in response to the relief of nitrogen-limitation (glutamine pulse upshift) using 4-thiouracil pulse-chase labeling and RNA sequencing. We find that accelerated degradation contributes to the rapid repression of Nitrogen Catabolite Repression (NCR) transcripts. Surprisingly, the degradation rate of some mRNAs encoding enzymes involved in carbon metabolism are also accelerated in response to a nitrogen upshift. At the same time, we find evidence for stabilization of mRNAs encoding the enzymes of the GMP biosynthesis pathway and some components of ribosome biogenesis machinery. To identify regulators of accelerated mRNA degradation, we screened for factors that impair accelerated degradation of GAP1 mRNA - a highly abundant transcript that is subject to NCR control. To accomplish this we developed a novel method integrating mRNA FISH, FACS, and sequencing (FFS) of molecular barcodes in a highly-multiplexed pooled screen of the prototrophic yeast deletion collection. We identified factors involved in mRNA quality control that contribute to this accelerated degradation of GAP1 mRNA, suggesting a possible role for mRNA quality control in mediating post-transcriptional remodeling of the transcriptome in dynamic environments.

Keywords: *mRNA dynamics, mRNA degradation, mRNA regulation, high-throughput screen, nitrogen-upshift, NCR, GAP1*

[PS8-7] Casein Kinase I protein Hrr25 is a Positive Regulator of Mitochondrial Biogenesis by Phosphorylating and Inhibiting Puf3 in S. cerevisiae

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Mitochondrial biogenesis in *S. cerevisiae* requires coordinated expression of genes encoding mitochondrial proteins. This is achieved in part via transcriptional control by the Hap2/3/4/5 complex and post-transcriptional control by Puf3. Puf3, a Pumilio RNA-binding domain protein, binds to the 3' untranslated region of a large number of mitochondrial protein-encoding mRNAs, leading to their decay and/or translational inhibition. The Puf3 binding motif is the sequence UGUAA(or U)AUA, with a C often found at the -2 position. Puf3 is a multiply phosphorylated protein and its hyperphosphorylation correlates with increased mitochondrial biogenesis, suggesting that increased Puf3 phosphorylation leads to its inactivation. The kinase responsible for Puf3 phosphorylation is unknown. Here we show that the casein kinase I protein Hrr25 negatively regulates Puf3 by promoting Puf3 phosphorylation. *hrr25* mutant cells have growth defects on medium with nonfermentable carbon sources, which can be reversed by a *puf3* deletion mutation. Hrr25 interacts with Puf3 and an *hrr25* mutation results in reduced phosphorylation of Puf3. We further found that an *hrr25* mutation reduces GFP expression from GFP reporter constructs carrying the 3' UTR of Puf3 targets. Down-regulation of GFP expression due to the *hrr25* mutation can be reversed either by a *puf3* deletion mutation or by mutations to the Puf3 binding sites in the GFP reporter transcripts. Together, our data indicate that Hrr25 is a positive regulator of mitochondrial biogenesis by phosphorylating and inhibiting Puf3.

Keywords: *Casein kinase I, Hrr25, Puf3, Mitochondrial biogenesis, Phosphorylation, 3' UTR*

[PS8-8] Nuclear Translocation of Hap Complex Components Required for Methanol-Regulated Gene Expression in the Methylotrophic Yeast Candida boidinii

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Methylotrophic yeasts, such as Pichia pastoris (Komagataella pastoris), Hansenula polymorpha (Ogataea polymorpha), and Candida boidinii, can utilize methanol as the sole source of carbon and energy. These yeasts have strong methanol-inducible gene promoters and have been used as hosts for heterologous gene expression systems. To achieve efficient protein production by methylotrophic yeasts, it is important to elucidate the molecular basis of transcriptional regulation of methanol-inducible genes in these yeasts. To date, we have identified several transcription factors required for methanol-regulated gene

expression (1). Recently, we identified genes encoding components of Hap complex, CbHAP2, CbHAP3, and CbHAP5 in C. boidinii (2,3). Although Hap complex in Saccharomyces cerevisiae is known to be essential for nonfermentable carbon metabolism, we found that CbHap complex is necessary for maximum activation of methanol-regulated genes and growth on methanol but not for the metabolism of other nonfermentable carbon sources. We also revealed that CbHap2p, CbHap3p, and CbHap5p localized to the nucleus and bound to the promoter regions of methanol-inducible genes regardless of the carbon source, and heterotrimer formation was suggested to be necessary for binding to DNA. Furthermore, nuclear localization of CbHap3p which has no nuclear localization signals (NLS) in its deduced amino acid sequence depended on CbHap5p but not on CbHap2p. In this study, in order to elucidate the mechanism for formation of CbHap complex, nuclear translocation of each component was investigated by using mutants in which putative NLSs was deleted or mutated. A proposed mechanism for nuclear translocation and complex formation of CbHap complex will be discussed.

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Keywords: *Hap complex, nuclear translocation, methylotrophic yeast*

[PS8-9] Regulation of GAL Gene Induction by Tor Signaling.

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The GAL genes in S. cerevisiae are highly regulated at the transcriptional level. GAL transcription is activated by Gal4p in response to the availability of galactose. Gal4p activity is positively regulated by Cdk8-dependent phosphorylation, by an uncharacterized mechanism that becomes essential for GAL induction in yeast lacking the galactose inducer protein Gal3p. Null mutations of cdk8/ srb10, or mutation of the Cdk8-dependent phosphorylation site on Gal4p at S699, render gal3 yeast incapable of growing on galactose as the sole source of carbon. We exploited this phenotype in a genetic screen to identify mutants that prevent Cdk8-dependent GAL expression in the hopes of discovering regulators of Cdk8. We have isolated 18 complementation groups, termed the gal four throttle (gft) mutants. One mutant, gft1, was found to represent a recessive allele of hom3, which encodes a metabolic aspartokinase in the biosynthetic pathway for threonine and methionine. Characterization of gft1 revealed a defect in Tor signaling; strains defective for gft1/ hom3 are hyper-sensitive to rapamycin, and cause constitutive Gat1p nuclear localization. Additionally, sublethal concentrations of rapamycin were found to prevent Cdk8-dependent GAL gene expression, as do the gft mutants. Furthermore, null mutations of tor1 or tco89, encoding components of the TORC1 complex, also prevent GAL expression in gal3 yeast, and these genes are likely represented within the initial collection of gft

mutants. Interestingly however, Cdk8 kinase activity in vitro towards known substrates is unaffected by rapamycin treatment or the gft1/ hom3 mutations, despite that Gal4 phosphorylation is altered gft1/ hom3 strains in vivo. Further genetic analysis demonstrated that disruption of cdc55, encoding a regulatory subunit of the PP2A protein phosphatase downstream of Tor signalling suppresses the effect of gft1/ hom3 mutations on GAL expression. Given these observations, we hypothesize that the Cdk8-dependent phosphorylation of Gal4 is opposed by one or more protein phosphatases that are regulated downstream of Tor signaling. These results provide novel insight into how induction of transcription by a specific inducer can be modulated by global physiological signals.

Keywords: *Transcriptional Regulation, Tor Signaling, GAL Gene Induction, Srb10/Cdk8*

[PS8-10] "mRNA imprinting": Regulation of Gene Expression by Co-transcriptionally Associating Proteins

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The coordination between stages of the RNA life-cycle is important for proper gene expression. We hypothesized that the decision making process of whether to express a gene (or a gene family) and the extent of its expression involves communication between all stages. Moreover, we found that the fate of a certain mRNA is already determined during transcription, even before its synthesis is completed. We surmise that co-transcriptional binding of factors, "mRNA imprinting", affects the fate of many mRNAs.

We have ascertained a broad scope of over 100 proteins that associate with the mRNA during transcription. Some of which are known to associate with mature and cytoplasmic mRNA. Amongst these proteins are a number of splicing factors, capping and polyadenylation factors and factors know to mediate mRNA export. Interactions of some proteins change under different conditions or stresses, such as heat shock and starvation. In response to stress, several chaperones bind mRNAs during transcription. Hence, mRNA imprinting is a widespread phenomenon.

Keywords: *transcription, mRNA biogenesis, mRNA processing, mRNA transport, heat shock*

[PS8-11] The Vid30c, an E3 Ubiquitin Ligase, Impacts the Regulation of Glucose Derepression During the Adaptation of Saccharomyces cerevisiae to Non-fermentable Ethanol

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The brewer’s yeast Saccharomyces cerevisiae has the ability to grow on a range of different carbon sources. In the presence of glucose (the preferred carbon source), the metabolism of non-fermentable carbon sources such as ethanol, acetate, and glycerol is inhibited by transcriptional repression and targeted protein inactivation and degradation. For example,

the transcription of gluconeogenic and respiratory genes are repressed when abundant glucose is available. In addition, gluconeogenic enzymes such as fructose-1,6-bisphosphatase (FBPase) are ubiquitinated and degraded when glucose-starved cells are replenished with glucose. This phenomenon, known as glucose repression, is governed by a complex, tightly-regulated signaling network. Signaling pathways active in glucose abundant conditions, such as the Ras2/cAMP/PKA pathway, participate in the transcriptional repression of glucose-repressed genes by inhibiting the function of the Snf1 kinase, which in turn is responsible for the derepression of these genes when glucose is absent. Snf1 is active in the absence of glucose and functions by phosphorylating and inhibiting the transcriptional repressor proteins, such as Mig1, needed for the repression of glucose-repressed genes. The Vid30 complex (Vid30c) is an E3 ubiquitin ligase known for its involvement in the degradation of FBPase and the hexose transporter Hxt3 in different carbon conditions. Here, qRT-PCR analysis suggests that the Vid30c also impacts carbon metabolism via gene transcription. Specifically, the Vid30c is required for the derepression of glucose-repressed genes when glucose is absent. Additionally, through Western blotting and live cell fluorescence microscopy, we show that the Vid30c may exert its influence on transcription by affecting the subcellular localization and post-translational modification of glucose-regulated signaling molecules.

Keywords: *Mig1, Vid30c, Carbon regulation*

[PS8-12] Genetic Variation in Transcription Factor Binding Sites Underlying Nitrogen Assimilation Differences in Yeast Isolates

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The conversion of grape must into wine is a complex process carried by several microorganisms, yet the baker yeast, Saccharomyces cerevisiae, is the main responsible of conducting the alcoholic fermentation. Nitrogen, a crucial nutrient in the fermentation process, is often present in limited amounts in grape must. Understanding the genetic basis underlying nitrogen assimilation differences among yeasts strains requires bridging the gap between their genotype and phenotype. Recently, we have estimated the extent of Allele Specific Expression (ASE) in six F1 hybrids generated from crosses between isolates of the four major yeast lineages, i.e. Wine/European, North American, West African, and Sake. Large differences in ASE between isolates were found, particularly within the WE-SA hybrid, which suggested that cis variants broadly influence gene expression differences between phenotypically distant strains. Several genes showing ASE were associated with differences in predicted allele specific transcriptional binding (ASB). Interestingly, the hemizygotes carrying the WE allele for ASN1, which encodes for an asparagine synthetase, showed greater assimilation levels of aspartic and glutamic acid than hemizygotes carrying the NA allele. ASB analysis of the ASN1 promoter region pointed to a single nucleotide polymorphism at the binding site of the transcription factor UGA3 as a potential variant driving ASN1 expression differences between these two yeast strains. Uga3p has been implicated in nitrogen catabolism, representing a potential candidate to underlie the

phenotypic differences observed between allelic variants. Indeed, our preliminary results have shown that UGA3 influence nitrogen assimilation profiles of the WE strain. To further evaluate the role of UGA3 in nitrogen assimilation, we will survey genome wide differences in UGA3 binding and expression levels in WE and NA isolates. To do so, we will perform Chip-Seq and RNA-seq analyses on F1 hemizygote hybrids (WExNA) carrying either the UGA3WE or UGA3NA allele. UGA3 binding differences might play a broader role in the regulation of nitrogen assimilation in S. cerevisiae, which could partially explain nitrogen assimilation differences among winemaking adapted yeast isolates, such as those from the WE lineage, with non-fermenting wild isolates, such as those from the NA lineage.

Keywords: *Genetics, Nitrogen assimilation, Transcriptional regulation*

[PS8-13] Differential Regulation of Two Alternatively Spliced Forms of Gcr1 transcription factor in Saccharomyces cerevisiae

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In Saccharomyces cerevisiae, glycolysis is the most important pathway for carbohydrate metabolism. Genes involved in glycolysis are regulated by several transcription factors, among which Gcr1 acts as a key transcription factor by binding to the CT box in the promoters. Gcr2 binds and activates Gcr1, but they can also form homodimers, whose functions are not well understood. Alternative splicing of GCR1, having unusually long intron, generates multiple mRNA forms including an unspliced form, where an intronic start codon can be used as a translation start site. Previous study suggested that both of the two major Gcr1 forms generated from spliced and unspliced mRNAs might be necessary for proper cell growth. In this study, we generated strains only producing either the spliced or unspliced form of Gcr1 by genome editing using CRISPR-Cas9 system. Contrary to the previous study using plasmid-based expression of GCR1 forms, two strains did not show any growth defects compared with the wild type. However, GCR2 deletion or deletion of the leucine zipper (LZ) domain of Gcr1 involved in homodimerization, led to differential growth defects depending on the types of Gcr1, suggesting that the two Gcr1 proteins might be deferentially regulated by Gcr2 binding and homodimerization, respectively.

Keywords: *Gcr1, CRISPR-Cas9, alternative splicing, intronic start codon (ATG), glycolytic genes*

[PS8-14] Tandemly Repeated Copies of a 94 bp Region in AGT1 Promoter Give a Variety in its Expression Level

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Agt1p plays an important role in beer brewing, as it can transport maltotriose which is often only partially consumed during fermentation. It is encoded close to telomere, and some strain-dependent diversities have been reported within its promoter and ORF. We

investigated AGT1 expression in several ale yeast, and found that some strains expressed AGT1 at relatively higher levels, while maltose transporter, MALX1, at a relatively lower levels. We obtained several AGT1 promoter sequences by PCR from five ale and one lager strains, and analyzed them. It was found that a promoter, which was most strongly induced by maltose, had four tandemly repeated copies of a 94 bp region (R1) at 722 bp upstream from the initiation codon. Other promoters with a single copy of R1 were extremely weak. Lager yeast, Weihenstephan 34/70 had single copy of R1 in its Sc-type AGT1 promoter, and its expression was low. In R1, one MAL-activating binding site (MGCN[9]MGS), and one Gcr1p-binding site was found using Yeastract database. Gcr1p is a transcriptional activator of genes involved in glycolysis. Another diverse region (R2) in AGT1 promoter was about 150-bp upsteam from the R1 region. R2 region included multiple repeats (two to seven copies) of Mig1p biding site. Mig1p is a transcription factor involved in glucose repression. The strongest promoter harbored seven Mig1p binding sites. We modified the strongest promoter and examined the activity of the derivatives. When copy number of R1 was reduced from four to two, promoter activity decreased about by two thirds. Reduction of Mig1p binding sites in R2 region from seven to six unexpectedly weakened the promoter activity roughly by half. R2 region seemed to have a positive effect on promoter activity in some manner. All of tested ale strains were triploid. We examined diversity in the R1 copy number within each strain. The strain with the highest AGT1 expression seemed to have major promoters with two or four copies of R1 region and minor ones with more copies. Another strain with the second highest AGT1 expression was found to have promoters with one or two copies of R1, and miner different ones. To make matters complicated, the sequences of AGT1 ORF in the tested strains had some varieties, indicating that AGT1 is highly diversified among ale yeasts. Some strains contain only AGT1 ORF terminated by an insertion at the middle of ORF, whereas others had only functional ORF, or had both functional and non-functional ORFs.

Keywords: *Ale yeast, sugar transporter, AGT1, promoter*

[PS8-15] Regulation of SPS100 Protein Abundance by an Antisense RNA Correlates With a Switch in mRNA Isoform Abundances

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Pervasive transcription of genomes generates, among others, also non-coding RNAs which overlap coding genes in antisense direction, termed antisense RNAs (asRNAs). The function of many asRNAs is not fully understood but several cases demonstrated their involvement in gene expression regulation of the overlapping sense gene. Previously we selected > 150 yeast genes with annotated asRNAs and tested them on the antisense-dependent regulation under 4 growth conditions. Using high throughput yeast genetics and unique system of unidirectional terminators we reported a regulatory function for approximately 25% of

asRNAs, most of which repress the expression of the sense gene. In this study we further explored the roles of asRNAs by testing more growth conditions and identified 15 conditionally antisense-regulated genes, where 8 of them were not previously reported to be regulated by antisense. Surprisingly, 6 of those genes exhibited antisense-dependent enhancement of gene expression, which is a rare case in antisense-dependent regulation. One of the genes positively regulated by asRNA was SPS100, which becomes upregulated upon entry into starvation or sporulation as a function of the antisense transcript SUT169. We demonstrate that the antisense effect is mediated by its 3' intergenic region (3'-IGR) and that this regulation can be transferred to other genes. Detailed functional analysis of the 3'-IGR revealed that Sps100 protein expression is highly dependent on the length and stability of SPS100 mRNA isoforms. The presence of SUT169 favors predominantly long and stable species from a predominantly short and unstable isoform. These results suggest a novel mechanism of antisense-dependent gene regulation via mRNA isoform switching.

Keywords: *pervasive transcription, antisense, regulation of transcription, mechanism*

[PS8-16] Protein Ubiquitination Affects Gene Expression Mediated by the Cell Wall Integrity MAPK Pathway in Yeast

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Living cells must be able to sense and respond properly to environmental stimuli that can affect their viability. In this regard, signaling pathways mediated by mitogen-activated protein kinases (MAPKs) play an essential role. In Saccharomyces cerevisiae, the cell wall integrity MAPK pathway (CWI) is the main signaling route responsible for controlling the adaptive response to conditions that perturb the cell wall (1). In this situation, yeast cells trigger a transcriptional response dependent on the MAPK Slt2 and the transcription factor Rlm1 in coordination with the SWI/SNF chromatin remodeling complex (2) and SAGA histone-modifying complex (3).

In the last years, protein modification by ubiquitination has emerged as an important regulator of signal transduction pathways (4). Ubiquitination is carried out by a cascade of enzymes, namely E1 (an ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase), whose result is the transfer of a single or several ubiquitin molecules to a specific substrate. Ubiquitination is a reversible process mediated by deubiquitinating enzymes (DUBs). Usually, polyubiquitynation of proteins implies their degradation by the proteasome, while multi- or monoubiquitylation is related to intracellular trafficking, mRNA transport, DNA repair, chromatin dynamics and, transcription (5). In the case of CWI pathway, ubiquitination processes regulate the activity and stability of two of its components, Pkc1 (6) and Rho1 (7), but the relation between ubiquitination and transcriptional regulation under cell wall damage conditions is still uncharacterized.

In this work, we have identified several elements of the ubiquitination/deubiquitination machinery potentially involved in the regulation of the gene expression associated to cell wall stress mediated by the CWI pathway. Specifically, two of them, Rad6 (E2 enzyme) and Dia2 (E3 enzyme), are responsible for ubiquitination of proteins at ORF of CWI-responsive genes in a Slt2 dependent manner, which is necessary for an appropriate expression of these genes.

Keywords: *Ubiquitination, CWI pathway, Transcription, Cell wall stress*

[PS8-17] RPL22 of Kluyveromyces lactis Complements the Role of Rpl22A in Maintaining the RPL22 Paralog Ratio in Saccharomyces cerevisiae

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The ancestor yeast of the Saccharomyces lineage underwent a whole genome duplication (WGD). Most of the duplicated genes were lost, but ~10% were retained and diverged. It is believed that these genes provided new opportunities for the evolution of Saccharomyces. Ribosomal protein genes (RPGs) are prominent among the retained duplicated groups. RPGs exist mostly as pairs of paralogs and recent studies documented the existence of inter-genic regulatory circuitries controlling their relative transcript concentrations. One example of such relationship is the RPL22A/B pair, which codes for one of the mostly diverged paralogs.

To further characterize the mechanism of inter-genic regulation of RPL22, we used a panel of strains with various combinations of RPL22 intron and gene deletions. While the RPL22A/B mRNA ratio was 92/8 in the WT, it was 60/40 in the RPL22A/rpl22bΔi strain. The RPL22B mRNA was almost absent in the rpl22aΔi/RPL22B strain. Importantly, the mRNA levels were reflected also in protein abundancies. Splicing efficiency analysis of recombinant splicing reporters containing endogenous introns revealed that Rpl22 proteins but not their RNA binding mutants can block splicing when in excess and that this inhibition is more effective for the RPL22B intron. Both Rpl22A/B proteins interacted in a yeast 3H system with RPL22B intronic region between bp 165 and 236. The introns maintained their inhibitory potential in vivo when swapped between A and B genes. We used RNA-Seq analysis to map transcriptome responses to changed levels of Rpl22 proteins. The decrease of total Rpl22 below ~20% produced a robust response, which likely reflected impaired ribosome biogenesis. The decrease of the A/B protein ratio (at ~physiological Rpl22 concentration) had almost no effect on the transcriptome, whereas its increase (in rpl22bΔ) led to the downregulation of a set of genes involved in rRNA processing, ribosome biogenesis, and proteosynthesis. This observation points to a paralog-specific role of Rpl22B protein.

The single RPL22 gene of Kluyveromyces lactis, which did not undergo WGD, also responded to inhibition by Rpl22 when introduced into S. cerevisiae. KlRpl22 was able to bind RPL22B intron as well as inhibit mRNA production of both RPL22 paralogs. We

propose that the propensity of Rpl22 to bind certain types of RNA structures led to the establishment of an auto regulatory loop. In the duplicated genome, the relationship became inter-genic and asymmetric.

Keywords: *RNA processing, ribosomal protein genes, splicing, paralogs*

[PS8-18] Regulation of Gene Expression by lncRNA in Differentiated Surface and Invasive Cells of Wild Yeast Biofilm Colonies

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Long non-coding RNA (lncRNA) regulates expression of important yeast genes, such as FLO11 and IME1 and groups of lncRNA are stabilised under specific conditions, such as meiosis or stress. Here, we address the question of whether lncRNAs may be involved in metabolic differences between surface and little-understood invasive cell subpopulations of complex wild yeast biofilm colonies. We therefore extracted RNA from surface and invasive cells, prepared stranded cDNA libraries and carried out RNA-seq and differential expression analysis to compare the expression of genes and associated lncRNA transcripts in the two cell population. Upregulated lncRNA transcripts in surface cells are enriched for meiotic unannotated transcripts (MUTs), consistent with degradation of MUTs by the 3‘-5’ RNase, Rrp6p in invasive cells and degradation of Rrp6p in surface cells. We identified over 30 antagonistically regulated lncRNA/gene pairs involving surface cell-upregulated genes (with roles in processes such as meiosis/sporulation, alternative sugar metabolism and protein degradation) and 80 pairs involving genes upregulated in invasive cells (with roles in processes such as translation, purine/pyrimidine or amino acid metabolism and transport). Putative examples of negative regulation of gene expression in one sub-population of biofilm colonies by lncRNA include reduced expression of FAR1 (thus relieving mitotic cell cycle arrest) in sub-surface cells and reduced expression of the cyclin genes, CLN2 and CLN3 in meiotic surface cells (Wilkinson et al., manuscript in preparation).

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Keywords: *Biofilm, Colony, Yeast, RNA-seq, lncRNA*

[PS8-19] Slt2 MAPK as a Component of the Transcriptional Machinery under Cell Wall Stress

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The cell wall is a fungal essential structure that determines cell shape and integrity, making necessary efficient adaptive responses that guarantee cell survival. In the yeast Saccharomyces cerevisiae this response is mainly regulated through the cell wall integrity (CWI) pathway coordinated by the MAPK Slt2. Phosphorylation of Slt2 leads to the activation of the transcription factors SBF (Swi4/Swi6) and Rlm1, being Rlm1 responsible for most of the transcriptional output of the CWI pathway upon cell wall stress. In this work we elucidate a role for Slt2 as part of the transcriptional activation machinery that regulates gene expression through Rlm1. We show that, under cell wall stress, Slt2 is recruited to promoters and travels to coding regions of CWI Rlm1-dependent genes. To this end, phosphorylation by Mkk1/2 and Slt2 kinase activity are required. This binding is also dependent on Rlm1, Swi3 and Gcn5 proteins. At initial steps of transcription, the catalytic activity of Slt2 on Rlm1 is critical for the binding of the activator to promoters upon stress. Additionally, Slt2 itself acts as a transactivator being able to induce the transcription of CWI genes when it is bound to promoters by the Rlm1 DBD domain. In agreement with a role for the MAPK Slt2 as an integral component of the transcriptional complexes, Slt2 interacts with Pol II. On the other hand, the recruitment of Slt2 at coding regions suggests a possible role of this MAPK in elongation.

Keywords: *cell wall, transcription, Slt2, CWI pathway*

[PS8-20] Ccr4p-mediated Deadenylation is Critical for Survival of Differentiated Yeast Colonies

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A yeast colony is an example of a multicellular structure formed by a unicellular microbe. It has been previously shown that yeast cells within the colony can differentiate depending on environmental conditions to various cell types (Palkova et al. 2014, FEMS Yeast Res 14, 96-108; Piccirillo et al. 2015, Genetics 201, 1427-38). For example, when growing on non-fermentable carbon sources, the aging colony differentiates into an upper layer formed by U cells and lower layer formed by L cells. These two cell types substantially differ in many physiological, morphological and biochemical parameters (Cap et al. 2012, Mol Cell 46, 436-48; Cap et al. 2015, Cell Cycle 14, 3488-97). A flow cytometry based screen revealed strains defective in colony differentiation into U and L cells and survival of the differentiated cell types. Among the screened strains, ccr4delta demonstrated one of the most striking phenotypes with massive cell death occurring in U cells and only minor effect observed in L cells. Ccr4p is a major cellular mRNA deadenylase and a component of the essential CCR4-NOT complex which plays a wide spectrum of regulatory roles on different levels of gene expression and is conserved from yeast to humans. Deletion of other components of the CCR4-NOT complex did not exhibit similar phenotype to ccr4delta with the exception of the pop2delta strain lacking the other deadenylase of the CCR4-NOT complex which has a phenotype, similar to but less pronounced than ccr4delta. We concluded that mRNA deadenylation could be a process, critical specifically to survival of U cells but not L cells. To gain molecular insight into this phenomenon process we analyzed long and short-tailed

mRNAs on a genome-wide level using microarrays in wild type and ccr4delta colonies with the aim of identifying specific mRNAs differentially polyadenylated in ccr4delta. This work has been supported by GACR 15-08225S.

Keywords: *deadenylation, yeast colony, differentiation, CCR4-NOT*

[PS8-21] Cytoplasmic Deadenylases Ccr4 and Pop2 are Required for Translational Repression of LRG1 mRNA in the Stationary Phase

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Ccr4 and Pop2 are the subunits of the Ccr4-Not complex involved in mRNA poly(A) tail shortening in Saccharomyces cerevisiae. We have previously shown that Ccr4 and Pop2 negatively regulates expression of LRG1 mRNA encoding a GTPase-activating protein for the small GTPase Rho1, a component of cell wall integrity pathway, and deletion of LRG1 suppresses the temperature-sensitive growth defect of the ccr4Δ and pop2Δ mutants. We have also shown that the slow growth of the ccr4Δ and pop2Δ mutants is suppressed by deletion of another gene, PBP1, encoding a poly(A)-binding protein (Pab1)-binding protein 1; however, the underlying mechanism still remains unknown. In this study, we investigated how ccr4Δ, pop2Δ, and pbp1Δ mutations influence on the length of poly(A) tail and LRG1 mRNA and protein levels during long-term cultivation. In the log-phase ccr4Δ mutant cells, LRG1 poly(A) tail was longer and LRG1 mRNA level was higher than those in the log-phase wild-type (WT) cells. Unexpectedly, Lrg1 protein level in the ccr4Δ mutant cells was comparable with that in WT. In the stationary-phase ccr4Δ mutant cells, LRG1 poly(A) tail length was still longer and LRG1 mRNA level was still higher than those in WT cells. In contrast to the log phase, Lrg1 protein level in the stationary-phase ccr4Δ mutant cells was maintained much higher than that in the stationary-phase WT cells. Similar result was obtained in the stationary-phase pop2Δ mutant. Loss of PBP1 reduced the LRG1 poly(A) tail length as well as LRG1 mRNA and protein levels in the stationary-phase ccr4Δ mutant cells. Our results suggest that Ccr4 and Pop2 regulate not only LRG1 mRNA level through poly(A) shortening but also the translation of LRG1 mRNA, and that Pbp1 is involved in the Ccr4-mediated regulation of mRNA stability and translation.

Keywords: CCR4, POP2, Poly(A) tail, Translational repression

[PS9] Translational and post-translational control

[PS9-1] Proteomic analysis of eIF5A mutant under ER stress conditions

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The translation factor 5A (eIF5A) is highly conserved in Archaea and eukaryotes and is essential for cell viability. Although it was initially suggested a function for eIF5A in the translation initiation, it was in the elongation step that eIF5A function was better demonstrated. Recent data from our research group revealed a possible role for eIF5A in the secretory pathway and endoplasmic reticulum (ER) translocation. In addition, it has been suggested an activation of the ER stress response upon eIF5A depletion in mammalian cells. Based on that, it was tested the behavior of a yeast eIF5A mutant in the presence of dithiothreitol (DTT) and tunicamycin, both ER stress inducing-drugs. Interestingly, the eIF5A mutant is sensitive to DTT and resistant to tunicamycin. So, to understand the mechanism which leads to difference in phenotypes, it was performed a large-scale proteomic screening with those ER stress inducing-drugs using the ORF-GFP collection of Saccharomyces cerevisiae crossed with the eIF5A mutant through the synthetic genetic array methodology. Fluorescence intensities from each individual colony were assayed using a scanning fluorimager to reveals the differential GFP expression. In general, the differentially expressed genes found in DTT treatment presented an enrichment for those involved in regulation and progression of cell cycle, otherwise the differentially expressed genes found in tunicamycin treatment presented an enrichment for those involved in vacuole organization, vesicle-mediated transport and microautophagy. In the presence of DTT, within the 461 differentially expressed genes, 101 are related with Protein Folding in ER and Unfolded Protein Response, while in tunicamycin treatment, within the 298 differentially expressed genes, 72 are involved on those processes. These contrasts may be associated with the differences in the mechanism of action of the drugs and they contribute to better understand this dual behaviour of eIF5A role on the ER stress.

Keywords: *eIF5A, translation elongation, proteomic, ER stress*

[PS9-2] Phosphorylation of Cth2 protein and its Grr1-dependent degradation are essential to maintain Cth2 proper levels required for optimal growth during the iron starvation

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Iron is an essential micronutrient for all eukaryotic organisms because it participates as a redox cofactor in a wide range of metabolic pathways. Despite its abundance, iron bioavailability for living organisms is highly restricted due to its low solubility at physiological pH. In response to iron limitation eukaryotic cells optimize iron utilization by repressing non-essential iron-consuming pathways and activating indispensable iron-dependent processes. In yeast Saccharomyces cerevisiae Cth2 protein, characterized by the presence of two Cx8Cx5Cx3Hx18Cx8Cx5Cx3H tandem zinc fingers (TZFs), binds to AU-rich elements (AREs) of many mRNAs encoding for iron-containing proteins, promoting their degradation and leading to a decrease in respiration and an increase in dNTP synthesis. Previous studies have demonstrated that yeast cells need to fine-tune the expression levels of Cth2 protein because its excess can be detrimental for growth. We show that Cth2 is a highly unstable protein phosphorylated at serine residues 65, 68 and 70 under iron-deficient conditions. Mutagenesis of these serine residues does not eliminate Cth2 targeted mRNA degradation function, but it increases Cth2 protein stability. The F-box E3-ligase protein Grr1 is crucial to tightly regulate Cth2 protein levels because Grr1 recognizes phosphorylated Cth2 protein and facilitates it degradation by the proteasome. Both mutagenesis of Cth2 serine residues and GRR1 deletion give rise to yeast cells with significant growth defects under iron deficient conditions, emphasizing the physiological relevance of regulating Cth2 protein abundance.

Keywords: *Cth2, Iron deficiency, Grr1, Phosphorylation, Degradation*

[PS9-3] Tma108, a putative M1 aminopeptidase, is a specific nascent chain-associated protein in Saccharomyces cerevisiae

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The discovery of novel specific ribosome-associated factors challenges the assumption that translation relies on standardized molecular machinery. In this work, we demonstrate that Tma108, an uncharacterized translation machinery-associated factor in yeast, defines a subpopulation of cellular ribosomes specifically involved in the translation of less than 200 mRNAs encoding proteins with ATP or Zinc binding domains. Using ribonucleoparticle dissociation experiments we established that Tma108 directly interacts with the nascent protein chain. Additionally, we have shown that translation of the first 35 amino acids of Asn1, one of the Tma108 targets, is necessary and sufficient to recruit Tma108, suggesting that it is loaded early during translation. Comparative genomic analyses, molecular modeling and directed mutagenesis point to Tma108 as an original M1 metallopeptidase, which uses its putative catalytic peptide-binding pocket to bind the N-terminus of its targets. The involvement of Tma108 in co-translational regulation is attested by a drastic change in the subcellular localization of ATP2 mRNA upon Tma108 inactivation. Tma108 is a unique example of a nascent chain-associated factor with high selectivity and its study

illustrates the existence of other specific translation-associated factors besides RNA binding proteins.

Reference:

Delaveau T., Davoine D., Jolly A., Vallot A., Rouvière J.O., Gerber A., Brochet S., Plessis M., Roquigny R., Merhej J., Leger T., Garcia C., Lelandais G., Laine E., Palancade B., Devaux F., Garcia M. Tma108, a putative M1 aminopeptidase, is a specific nascent chain-associated protein in Saccharomyces cerevisiae. Nucleic Acids Res 2016 Oct 14;44(18).

Keywords: *Translation, Specific ribosome partner, Nascent-chain associated factor*

[PS9-4] Defect in the GTPase Activating Protein (GAP) Function of eIF5 Causes Repression of GCN4 and HIS4 Translation and Sensitivity to Starvation

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In eukaryotes, the selection of open reading frame (ORF) on the mRNA is the key fundamental step carried out by the 40S ribosome along with Met-tRNAiMet and several translation initiation factors. The factor eIF5 plays critical role in maintaining the fidelity of AUG start codon selection by providing GTPase activating protein (GAP) function through its N-terminal domain (NTD) to hydrolyse the GTP into GDP and Pi by the eIF2 ternary complex. The eIF5-CTD is reported to take part in 48S assembly/post-assembly process and mutations in this region confer both Gcn– (general control non-derepressed) and Gcd– (general control derepressed) phenotype in a temperature sensitive manner. However, none of the mutations in the eIF5-NTD is known to associate with Gcn– and Gcd– phenotype and this domain is only implicated in GAP function, suggesting a predominantly regulatory function to this region. The hyper GTPase eIF5-G31R mutant at the NTD was originally isolated as a dominant Sui– (Suppressor of initiation codon) mutant and observed to be recessive lethal. Our results indicate that eIF5-G31R mutant confers sensitivity to 3-Amino-1,2,4-Trizole (3AT). The 3AT is a competitive inhibitor of HIS3p-enzyme and induced histidine starvation. Detail molecular analysis reveals the eIF5-G31R mutation is causing two distinct phenomena, one is related to the 3AT sensitivity while the other is related to the repression of GCN4 expression (Gcn– phenotype). The down regulation of GCN4 expression was not because of leaky or slow scanning, rather due to a novel mechanism that is linked with utilization of upUUG initiation codon present at the 5' regulatory region between uORF1 and the main GCN4 ORF. The sensitivity to 3AT was partially rescued by supplementing HIS4(UUG) allele, indicating a defect in the start codon recognition from HIS4(AUG) allele in eIF5-G31R mutant. eIF1 monitors codon:anti-codon interaction and increases the stringency of AUG start codon selection. Consistently over-expression of eIF1 rescues 3AT sensitivity of eIF5-G31R mutant in the presence of HIS4(AUG) allele and highlights the expression HIS4 could have been the limiting factor for histidine biosynthesis. Consistently, overexpression of HIS4(AUG) alleles rescues the 3AT sensitivity

in eIF5-G31R mutant. We are providing a possible mechanism of 3AT resistance confer by overexpression of HIS4p-enzyme which is not a direct target of 3AT.

Keywords: *Translation Initiation, eIF5, GCN4 expression*

[PS9-5] Temporal Post-Translational Modifications of Rpb4/7 Uncover a Novel Type of Regulation

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The Yeast proteins Rpb4 and Rpb7 form a dissociable heterodimer. Rpb4/7 plays roles in all the major stages of the mRNA life cycle: regulation of transcription, mRNA export, translation and mRNA decay. Rpb4/7 binds Pol II transcripts co-transcriptionally and then escorts the mRNA throughout its life. Previously, we found that it modulates each step that the RNA undergoes by its capacity to interact with key regulators temporally and spatially. We proposed that it can integrate all stages into a system (the "mRNA coordination" concept). Using 2-dimensional gel electrophoresis, here we show that Rpb4/7 exists in various proteoforms (~ 100 potential combinations). Using Mass Spectrometry, we found that these proteoforms contain post-translational modifications (PTMs) like N-terminal acetylation, phosphorylation, methylation, and ubiquitination or neddylation. This provoked us to examine whether these PTMs are involved in its capacity to integrate all stages into a system. We found that the PTMs change as Rpb4/7 progresses from one stage to the other. Specifically, in the context of transcribing Pol II, Rpb4/7 seems to be unmodified. Most modifications are dependent on prior binding to Pol II. These modifications are biologically significant because they are responsive to environmental conditions, such as heat shock and required for optimal cell proliferation at non-permissive temperatures. We show that certain Rpb4 PTMs affect the steady-state mRNA level and mRNA degradation rates of some genes. Lack of certain Rpb4 PTMs compromise Nonsense-mediated mRNA decay as well. We propose that PTMs play roles in quality controlling the various stages of gene expression and in the capacity of Rpb4/7 to function as an mRNA coordinator.

Keywords: *RNA Polymerase II, Post-translational Modifications, Regulation, Protein*

[PS9-6] A Putative Novel Saccharomyces cerevisiae Translation Factor Rescues the Lethality of eEF3 Loss

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In yeast, translation elongation requires a yeast-specific ABCF ATPase Elongation Factor 3 (eEF3) encoded by the YEF3 gene. The factor is essential for yeast viability, but is absent in other eukaryotes, such as humans. The reason for its essentiality is unclear. We have used

genetic suppression of yef3Δ cells to test protein factors that could overcome the essentiality of eEF3.

We describe a new putative translation factor that upon overexpression renders YEF3 knock-out strain viable, though the suppression is not perfect as manifested by pronounced growth defect. Cells lacking the putative factor are more sensitive to eEF3 depletion, suggesting that the two factors have overlapping functions in the cell under normal conditions. The protein co-sediments with ribosomes and addition of the factor promotes translation in cell-free extract made from the strain lacking the factor – but not in the lysates made from the wild type yeast. Conversely, a catalytically inactive form of the protein is locked on the 80S ribosome and potently inhibits translation. The specific biological function of the factor is yet to be determined.

Keywords: *eEF3, translation, ribosome*

[PS9-7] Co-ordinate regulation of the glycolytic pathway at the level of mRNA localisation in yeast

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mRNA localisation represents a means to control the translation, stability and overall fate of mRNAs. In recent work we have shown that two glycolytic mRNAs (PDC1 and ENO2) localise to the same discrete granules in actively growing yeast cells. This raises the question as to whether these granules can serve as a factory for the production of proteins from the same metabolic pathway or protein complex. In order to explore this idea further, 20 other glycolytic mRNAs were investigated using the m-TAG technique and shown to localise to granules in live cells. Using ‘translating RNA imaging by coat protein knock-off’ (TRICK), we found that localisation of glycolytic mRNAs to the granules is linked to active mRNA translation, and that translation represents a prerequisite for mRNA localisation to the granules. The propensity of glycolytic mRNAs to enter granules varies depending on nutrients, with highly fermentable carbon sources favouring granule localisation. Furthermore, we discovered that while a core of essential glycolytic mRNAs co-localise to the same granules, termed core fermentation- CoFe granules, the remaining mRNAs localise to a different class of granule called accessory fermentation- AFe granules. We hypothesise that this arrangement permits the production of different protein complexes involved in glycolysis. Overall, our data support the idea that mRNAs of the same metabolic pathway can be co-ordinately translated at specific sites and lead to the possibility that the regulation of these pathways could be orchestrated post-transcriptionally.

Keywords: *mRNA localisation, Translation, Glycolysis, RNA granules, Cytoplasm, Fermentation, Regulation of Gene expression*

[PS9-8] Readthrough Inducing Potential of Glutamine Isoacceptor tRNAs Lies in Their Primary Sequence Outside of the Anticodon

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Glutamine was repeatedly observed to incorporate almost exclusively into a few viral and cellular polypeptides during programmed stop codon readthrough. In some other cases, however, almost no incorporation of glutamine was detected. Here we asked what factors are responsible for the selective incorporation of the glutamine amino acid in these observed cases. Our recent findings suggested that one specific glutamine isoacceptor tRNA, bearing the CUG anticodon, manifests a great readthrough potential at the UAG-N stop tetranucleotide when compared to the low readthrough potential of all other tested glutamine isoacceptors bearing the UUG anticodon instead to base pair with the UAA stop. Interestingly, all glutamine isodecoder genes are sequentially otherwise very similar varying only in up to 6 nucleotides. We took a closer look at a contribution of the differing anticodon versus single nucleotide changes in the primary sequence of the Glu-tRNA backbone with respect to their varying readthrough potential. Strikingly, we observed that the backbone of glutamine tRNA (tQ(CUG)M) is the major contributor to its high readthrough potential and thus it could be used as an exceptionally potent readthrough inducing tRNA (rti-tRNA) also for UAA and UGA stop codons when its anticodon is mutated to UUG or C/GCA, respectively. Our comprehensive analysis of the readthrough inducing potential of glutamine tRNAs hence brings new insights into stop codon decoding with the unexpected role of the tRNA structure in this process. It is also noteworthy that this still ongoing development of a “superpower” rti-tRNA could have a great application in a medical research by contributing to the common effort of many labs to specifically increase readthrough at premature termination codons, which are the major cause of more than 15% of genetic diseases and inherited cancers.

Keywords: *tRNA, Translational Readthrough, Genetic Diseases and Inherited Cancers*

[PS9-9] Combinatorial Library Strategy for Secretory Overexpression of the Thermostable Lipase in Saccharomyces cerevisiae

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Saccharomyces cerevisiae can produce heterologous proteins extracellularly and efficiently. Promoters, secretion signals, and terminators modulate transcription initiation, transportation of proteins after translation, and transcription termination, respectively. Thus, selection of these elements is important for the secretory expression of proteins. In this study, a combinatorial library strategy was used to improve the secretory expression of a lipase from Bacillus thermocatenulatus (BTL2) in the culture supernatant of Saccharomyces cerevisiae. A plasmid library including expression cassettes composed of sequences encoding 15 promoters, 15 secretion signals, and 15 terminators derived from yeast species, S. cerevisiae, Pichia pastoris, and Hansenula polymorpha, was constructed

and transformed into S. cerevisiae. Using high-throughput screening of transformants using medium plates and 96-well plates, we successfully obtained yeast transformants that showed high lipase activity. Among approximately 14,000 transformants, the transformant YPH499/D4, comprising H. polymorpha GAP promoter, S. cerevisiae SAG1 secretion signal, and P. pastoris AOX1 terminator, was selected. This transformant expressed BTL2 extracellularly with a 130-fold higher than the control strain, comprising S. cerevisiae PGK1 promoter, S. cerevisiae α-factor secretion signal, and S. cerevisiae PGK1 terminator, after cultivation for 72 h. This combinatorial library strategy holds promising application in the optimization of the secretory expression of proteins in yeast.

Keywords: *overexpression, Saccharomyces cerevisiae, combinatorial library strategy, lipase*

[PS9-10] Eukaryotic translation initiation factor 3 undergoes dramatic structural changes prior to its binding to the 40S ribosomal subunit

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Eukaryotic translation initiation factor 3 (eIF3) is a multiprotein complex serving as an essential scaffold promoting binding of several other factors to the 40S subunit, where it coordinates their actions during the initiation phase of translation. It comprises 12 proteins in mammals, whereas only 5 subunits in the budding yeast. Due to a high degree of flexibility of multiple eIF3 subunits/domains, a detailed crystal structure of neither mammalian nor yeast eIF3 complexes has been solved till now. However, high-resolution models of eIF3 in complex with the 40S subunit plus some other eIFs were published based on short-length cross-linking, negatively stained EM reconstruction and cryo-EM imaging. We previously examined the protein-protein interactions among yeast eIF3 subunits and based on these biochemical experiments supported by various genetic analyses we built the eIF3 subunit interaction map, where we inserted all known crystal structures of eIF3 subunits or their domains. Here we purified all individual subunits of yeast eIF3 from bacteria and reconstructed the whole protein complex in vitro to determine an overall shape of the free 5-subunit eIF3 in solution. The reconstituted eIF3 was cross-linked with four different cross-linkers and the trypsin-digested samples were analyzed using Mass spectrometry. The obtained cross-links not only support our aforementioned eIF3 subunit interaction map but also reveal a completely new geometry of eIF3. The whole complex seems to be very compactly packed when free in solution, which contrasts with the published eIF3-40S models, where eIF3 appears to wrap around the 40S head with its extended arms. To understand the process of the eIF3 rearrangement/unpacking during Multifactor complex (eIF3, eIF1, eIF5, and eIF2 ternary complex) formation and its binding to the 40S subunit, we analyzed the eIF3-eIF1-eIF5 complex as well as the eIF3-40S preinitiation complex using the same methodology. The obtained results support our idea that binding of other factors slightly opens compactly packed eIF3 complex prior to its binding to the 40S subunit, where it spreads its arms to literarily embrace it. Our data thus suggest a robust structural rearrangement of the overall shape of eIF3 prior to its 40S-binding with functional implications that will be discussed.

Keywords: *eIF3, Translation initiation, Cross-linking coupled with mass spectrometry*

[PS9-11] Looking for RPE ii. partner in GCN4 translation reinitiation

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Translation reinitiation (REI) is a gene-specific control mechanism characterized by the ability of some short upstream ORFs to prevent recycling of the post-termination 40S subunit in order to resume scanning for reinitiation downstream. Yeast GCN4 mRNA contains four short uORFs in its 5' leader, out of which the first two (uORF1 and uORF2) are highly REI permissive while the remaining two (uORF3 and uORF4) pose a barrier to GCN4 translation.

Previously we have demonstrated that the high REI competence of uORF1 and uORF2 depends on several cis-acting features out of which the REI-promoting elements (RPEs) create a specific structural arrangement (5' enhancer) in their upstream regions. uORF1 utilizes four RPEs (i.–iv.), whereas uORF2 separately utilizes only a single RPE v. (similar in sequence with the uORF1-specific RPE i.) and, in addition, shares the function of RPE ii. with uORF1. We have also revealed that, in order to retain 40S subunit on mRNA upon termination, RPE i. and RPE iv. of uORF1 and RPE v. of uORF2 co-operate with the extreme N-terminal domain of the a/TIF32 subunit of the eukaryotic initiation factor 3 (eIF3). In our most recent work we provided direct in vivo evidence of these interactions and also proved the long-standing hypothesis that eIF3 is one of the initiation factors preserved on the 80S ribosomes during early elongation of translation, an essential precondition for its role in REI. However, the functions of remaining RPEs (RPE ii. and iii.) in REI are still not known. Here we concentrated on finding the interacting partner(s) of RPE ii., a small stem-loop structure which makes critically important contribution to REI promoted by both permissive uORFs. We used in vitro transcribed RNAs containing wild-type and mutated segments of GCN4 5' enhancer as baits in RNA-protein pull-down experiments and assayed cellular extract prepared from S. cerevisiae under various conditions. The subsequent mass-spectrometry analysis revealed a number of potential RPE ii.-binding candidates with functional relation to translation machinery which were further analysed by genetic and biochemical testing. Determination of molecular roles of all functional elements regulating the GCN4 expression will provide important information towards elucidation of the molecular mechanism of translational reinitiation the nature of which is largerly still mysterious.

Keywords: *translation reinitiation, short upstream ORF, GCN4 transcript leader sequence, RNA-protein interaction, regulation of gene expression*

[PS9-12] Variation of the eRF3 translation termination factor in Saccharomyces yeasts

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In eukaryotes, a number of proteins counteract to release nascent polypeptide from the ribosome, the eRF1 and the eRF3 translation termination factors being the most profoundly studied of them. In yeast, these factors are encoded by the SUP45 and SUP35 genes, respectively. A lot of sup45 and sup35 mutations affecting different functions of eRFs were obtained in laboratory, but little is known about natural variation of these genes. Epigenetic factors such as Sup35p conversion into [PSI+] prion may affect translation termination efficiency, so it is tempting to look for the SUP35 alleles naturally prone to shift this character. We retrieved all SUP35 sequences available so far for different Saccharomyces yeasts strains in Genbank and SGD and compared them. No natural substitution in this gene matches laboratory mutations known to affect eRF3 function. We found no obvious traces of recent natural selection in SUP35. We identified several substitutions or indels specific for different groups of strains or yeast species. These results indicate that translation termination factors are indeed conservative at the sequence level.

The work was supported by grants of President of Russian Federation (Nsh-9513.2016.4) and of Saint Petersburg State University (1.37.291.2015 and 1.41.1130.2017).

Keywords: *translation termination, nonsense suppression, evolution*

[PS9-13] The small ribosomal protein RPS3 and its functions

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Eukaryotic translational initiation requires interaction of many protein factors with the 40S ribosomal subunit. These factors are called eukaryotic initiation factors (eIFs) and numerous studies have been published over the years describing their specific roles not only in translational initiation, but also in termination and ribosomal recycling. Small ribosomal proteins are key players in anchoring the eIFs to the ribosomal surface and perhaps even in modulating their function, but their exact roles remain largely unexplored. In this study, we focused on small ribosomal protein RPS3, which lies near the mRNA entry channel and is known to be a part of the latch mechanism modulating mRNA recruitment and movement of the ribosome along mRNA. In addition, RPS3 interacts with the TIF32 and TIF35 subunits of eIF3, recently implicated in controlling translation termination and promoting programmed stop codon readthrough. Indeed, our experiments identified two RPS3 variants with altered readthrough levels. Interestingly, both mutants seem to have an opposite effect on the efficiency of readthrough (increase vs. decrease), which most likely reflects their specific orientation towards other constituents of the latch mechanism.

Keywords: *RPS3, readthrough, translational termination*

[PS10] Protein and mRNA quality control – from translation to stress response

[PS10-1] Heat Shock Stress Granules Dissolution in Saccharomyces Cerevisiae – A Question of Life and Death?

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In response to various types of stress general translation in cells is either attenuated or even completely arrested not only for energy savings and prevention of synthesis of unnecessary proteins but probably also to enable a better cell survival. Components of the translation machinery then accumulate in foci called stress granule (SGs) whose composition varies depending on the type and robustness of the applied stress and the organism. The role of SGs is not fully understood, but it seems that SGs dissolution is a key step in successful cell recovery.

We have first described heat stress-induced SGs in Saccharomyces cerevisiae formed upon robust heat shock (HS) containing components of translation initiation, elongation, and termination complexes as well as many other proteins including those involved in the mRNA metabolism. Here we present an analysis of the influence of the autophagy machinery and other proteins including heat shock proteins on the dissolution of HS-induced SGs. Our results from GFP-Atg8 processing assay demonstrate that upon robust heat shock (10 minutes at 46 °C) there is no autophagy induction. Further, our analysis of an autophagy defective strain (Δatg1 deletion mutant) indicates that there is even faster SGs dissolution compared to the wild-type strain when autophagy is absent. This suggests that autophagy does not take part in the dissolution of HS-induced SGs what is in sharp contrast with the dissolution of SGs induced by glucose depletion in S. cerevisiae that is autophagy dependent. Our further results confirm that the heat shock protein Hsp104 is really a key player in HS-induced SGs dissolution. Upon absence of Hsp104, the cell population does not resume the growth like the wild-type strain does and the majority of cells in the heat-shocked population is subjected to apoptosis. The reason being the persistence of HS-induced SGs. This suggests that successful dissolution of SGs is essential for cell recovery and stress survival.

This work was supported by the grant from the Czech Science Foundation CSF16-05497S.

Keywords: *yeast, heat shock, stress granules, dissolution, cell recovery*

[PS10-2] Isolating Mistranslating Serine tRNAs With a Proline Anticodon Through an Ambivalent Intermediate

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The genetic code is the language that converts genetic information into protein sequence. Though generally believed to be fixed, many examples indicate that variations to the code provide a selective advantage. In this study, we have used a sensitive selection system involving suppression of tti2-L187P to detect mistranslation in S. cerevisiae and study mechanisms that result in altered use of the genetic code. A serine tRNA containing a proline anticodon UGG [tS(UGG)] is toxic in yeast, likely because of excessive mistranslation. However, we selected four variants of this tRNA that caused incorporation of serine at proline codons. Each contained a single mutation in the tRNA. One mistranslating tS(UGG) variant decreased the growth rate as indicated by a 1.4-fold increase in doubling time as compared to the wild-type strain, induced a heat shock response and was lost rapidly in the absence of selection. Of note, the secondary mutation in the context of an otherwise wild-type tS(UGA) gave no phenotype. A second variant had minimal effect on cell growth, was relatively stable in cells and gave rise to a partial heat shock response. We hypothesize that mistranslating tRNAs with anticodon switches can evolve through an “ambivalent intermediate” – a mutation that partially reduces the activity of the tRNA. Due to the high copy number of tRNA genes, these ambivalent intermediates do not result in a phenotype and therefore are not selected against. Importantly, they provide the cell with a tRNA primed for non-toxic levels of mistranslation upon acquiring a mutation to the anticodon. If this mistranslation provides a selective advantage, it can lead to genetic code evolution.

Keywords: *tRNA, Mistranslation, Genetic Selection*

[PS10-3] Budding yeast HECT_2 protein Ipa1 is an essential ubiquitin-protein ligase influencing proteasome activity

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The ubiquitin-proteasome system (UPS) controls cellular functions by maintenance of a functional proteome and degradation of key regulatory proteins. Central to the UPS are ubiquitin-protein ligases that selectively catalyze modification of target proteins with ubiquitin, thereby controlling their abundance or initiating regulatory events. Here, we demonstrate that the essential Saccharomyces cerevisiae protein Yjr141w/Ipa1 (Important for cleavage and PolyAdenylation) is a HECT_2 (homologous to E6-AP carboxyl terminus_2) -type ubiquitin-protein ligase. We identified residues within the HECT_2 family signature that are essential for Ipa1 function and provide evidence for the importance of the C-terminal region, which has low similarity to HECT-type ubiquitin-protein ligases. In agreement with a function as ubiquitin-protein ligase, Ipa1 shows interactions with several ubiquitin-conjugating enzymes in vivo and exhibits autoubiquitylating activity in vitro. The activity of the nuclear localized Ipa1 is important for cell cycle progression and cell size control. Loss of Ipa1 function reduces proteasomal degradation of several substrates and results in activation the Rpn4 regulon. Our results demonstrate that Ipa1 is an ubiquitin-protein ligase, which influences proteasome activity in yeast.

Keywords: *Ubiquitin proteasome system, Protein degradation, Ubiquitin-protein ligase*

[PS10-4] COX1 Gene on Mitochondrial DNA Contributes to High Hydrostatic Pressure Stress Response

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High hydrostatic pressure (HHP) is one of the physical stresses, same as a temperature, and causes inactivation of microorganisms. However, there is little report concerning HHP stress response as compared with heat shock response. Therefore, we isolated pressure-sensitive (piezosensitive) mutant strain and evaluated by a genomic technology.

The piezosensitive strain a924E1 was isolated from a Saccharomyces cerevisiae strain KA31a using UV mutagenesis. We characterized the genetic factors causing piezosensitivity by DNA microarray. The gene expressions of 498 and 649 genes of strain a924E1 were higher and lower, respectively, than those of strain KA31a. Many genes, which showed altered expression levels, were classified into the energy category and the proteins encoded by these genes were located in mitochondria. These results suggested that genes, encoding mitochondrial proteins related to energy metabolism, contributed to piezosensitivity.

The mitochondrial function of strain a924E1 was evaluated by TTC staining test and it showed a negative response, which suggested mitochondrial dysfunction. We analyzed the inheritance of these phenotypes using diploid strains obtained by mating strain a924E1 and a wild type strain. The resultant diploid strains exhibited 1 of 2 phenotypes of TTC-positive or TTC-negative. Each group showed piezotolerance or piezosensitivity, respectively. These results suggested that the piezosensitivity was due to mitochondrial DNA (mtDNA) because these phenotypes were cytoplasmic inheritance. The results of PCR amplification to identify genetic alterations causing the piezosensitivity, some ORFs were able to be detected, however, the region containing the COX1 gene was not detected. Thus, we concluded that the piezosensitivity was associated with a mitochondrial dysfunction caused by deletion of COX1 gene.

However, direct evidence causing the piezosensitivity was not revealed because knockout of mtDNA has not been established. We are considering induction of the piezosensitivity into a wild type strain by knockout of COX1 gene using CRISPR/Cas9 system.

This research was supported by grants from the Project of NARO Bioriented Technology Research Advancement Institution (the special scheme project on regional developing strategy).

Keywords: *high hydrostatic pressure, Saccharomyces cerevisiae, mitochondria, DNA microarray*

[PS10-5] Metals and Metalloids Cause Protein Misfolding and Aggregation

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Certain metals and metalloids, such as cadmium and arsenite, are common environmental pollutants. They are considered highly toxic and are classified as human carcinogens. While their toxicity is undisputed, the underlying in vivo molecular mechanisms are not fully understood. Here, we demonstrate that cadmium, like arsenite, induces aggregation of cytosolic proteins in living yeast cells. Cadmium primarily targets proteins in the process of synthesis or folding, probably by interacting with exposed thiol groups in not yet folded proteins. Cells that cannot efficiently protect the proteome from cadmium-induced aggregation or clear the cytosol from protein aggregates are sensitized to cadmium. Thus, protein aggregation may contribute to cadmium toxicity.

Using a high-content imaging screen, we identified processes that control protein aggregation upon As(III) exposure. We demonstrate that blocking transcription during As(III) exposure prevents protein aggregation. Furthermore, we show that a loss in transcriptional control leads to enhanced protein aggregation and As(III) toxicity. Interestingly, the molecular chaperone Sse1p acts as buffer in cells that cannot properly regulate transcription already in the absence of As(III), but even more so during As(III) exposure. Altogether, our findings suggest that the loss of transcriptional control leads to accumulation of protein aggregates, thereby contributing to As(III) toxicity.

Keywords: *Metal toxicity, Protein aggregation, Protein folding*

[PS10-6] Genetic and environmental factors affecting the accuracy of translation in yeast

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It is essential for the cell to have correctly synthesized proteins and therefore the need to avoid erroneous translation is likely a key factor influencing the evolution of protein coding sequences. The actual accuracy of protein synthesis must depend on both genetic and environmental factors but the relative strength of the two factors remain unknown. We used a collection of Saccharomyces cerevisiae isolates and an array of environmental conditions to address this question. We measured the frequency of incorrect amino acid incorporation for two arginine codons, AGG – recognized by a rare tRNA and AGA – recognized by other and more common tRNA. We found that the rate of mistranslation depended chiefly on codon identity regardless of what strain or environment (benign or stressful) was used. Specifically, the AGG codon was invariably mistranslated at a higher

rate than the other codon. Another clear pattern was that each single strain grown under different conditions showed a strong positive correlation between the rate of growth and the rate of mistranslation. However, the strong within-strain dependence of the error rate on growth rate did not extend to the among-strain variation. Under standard conditions, individual strains differed up to three-fold in the rate of translational errors but this could not be explained by neither variation in the growth rate nor variation in the translation rate. By analyzing the DNA sequence of these strains, we noted that the rate of mistranslation is best associated with variation within genes associated with amino acid metabolism and homeostasis. We hypothesized that most critical would be the availability of arginine. Indeed, in subsequent experiments we demonstrated that the rate of mistranslation depended on the level of arginine in growth medium. Our results confirm that the availability of tRNA is a universal determinant of the accuracy of protein synthesis. But they also highlight the fact that not only the abundance of a carrier (tRNA) but also that of a cargo (amino acid) can be comparably important for a particular organism. These two factors, both dependent on genes, remain valid in different environments.

Keywords: *translation accuracy, amino acid homeostasis, yeast populations, stress*

[PS10-7] Physiological Regulation of Heritable Protein Aggregation

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Ordered protein aggregates (amyloids) and their transmissible variety (prions) are associated with important human diseases. In yeast, self-perpetuating protein isoforms, termed yeast prions, control heritable traits. Prion formation and loss are modulated by environmental and physiological conditions. Our data show that a heat-induced metastable prion, formed by an actin assembly protein, may persist in a fraction of yeast cells for a significant number of cell generations after return to normal growth conditions, thus carrying a cellular memory of stress. Moreover, evolutionary acquisition of a prion-forming ability by this protein coincides with the acquisition of increased thermotolerance in the Saccharomyces lineage (Chernova et al., 2017 Cell Reports 18: 751). Propagation of yeast prions is controlled by the same cytosolic chaperones that are responsible for the protection of yeast cells against a proteotoxic stress. Chaperones fragment prion polymers and generate new oligomeric seeds, promoting new rounds of aggregation. Therefore, yeast prions are adjusted to physiological levels of chaperone proteins and hijack a cellular stress defense machinery for their own propagation. Chaperones of the ribosome associated complex, that are involved in proper folding of a nascent polypeptide, antagonize initial prion formation. During a stress, the decrease in overall translational activity is accompanied by a relocation of the ribosome associated chaperones into a cytosol, resulting in an impairment of a prion-like propagation of misfolded proteins. Cellular apparatus, controlling the asymmetry of a mitotic division, influences maintenance and properties of

self-perpetuating protein aggregates both during recovery from stress and in the process of replicative aging. Overall, intimate relationship with the protein quality control machinery of the cell plays a key role in the processes of prion formation and propagation in yeast. (Supported by grants MCB 1516872 from NSF, and 14-50-00069 from RSF.)

Keywords: *Amyloid, Chaperone, Prion, Protein Quality Control, Stress*

[PS10-8] Arsenite Causes Protein Misfolding And Aggregation In Vivo

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Arsenic is a major environmental pollutant and chronic exposure is associated with cardiovascular abnormalities, neurotoxicity and with cancers of the skin, bladder and lung. This metalloid has also a long history of usage in medical treatment. Yet, the molecular mechanisms of its biological actions are not entirely clear. Here, we show that arsenite [As(III)] triggers protein aggregation in Saccharomyces cerevisiae . Proteins in the process of synthesis/folding are particularly sensitive to arsenite-induced aggregation in vivo. Aggregated proteins have high translation rates and are substrates of ribosome-associated Hsp70 chaperones, indicating that they are susceptible for aggregation primarily during translation/folding. Using a genome-wide high-content imaging screen approach, we identified yeast deletion mutants with either increased or decreased levels of protein aggregation. Those 127 mutants found to accumulate more aggregates than the wild type during As(III) exposure are enriched for functions related to cellular signaling, metabolism and protein folding/degradation. 168 deletion mutants accumulated less aggregates than the wild type and these mutants are enriched for functions related to protein biosynthesis and transcription. Our data show a correlation between mutants that are As(III) sensitive and mutants that hyper-accumulate protein aggregation during As(III) exposure. Similarly, there is a correlation between As(III) resistant mutants and mutants that accumulate few aggregates during exposure. These findings indicate that protein aggregation contributes to As(III) toxicity and the clearance of aggregates is important for tolerance.

Keywords: *Arsenic, Metal, Protein aggregation, Protein quality control*

[PS10-9] In vivo evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation

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Translation reinitiation is a gene-specific translational control mechanism characterized by the ability of some short upstream ORFs to prevent recycling of the post-termination 40S subunit in order to resume scanning for reinitiation downstream. Its efficiency decreases with the increasing uORF length, or by the presence of secondary structures, suggesting that

the time taken to translate a uORF is more critical than its length. This led to a hypothesis that some initiation factors needed for reinitiation are preserved on the 80S ribosome during early elongation. Here, using the GCN4 mRNA containing four short uORFs, we developed a novel in vivo RNA-protein Ni2+-pull down assay to demonstrate for the first time that one of these initiation factors is eIF3. eIF3 but not eIF2 preferentially associates with RNA segments encompassing two GCN4 reinitiation-permissive uORFs, uORF1 and uORF2, containing cis-acting 5' reinitiation-promoting elements (RPEs). We show that the preferred association of eIF3 with these uORFs is dependent on intact RPEs and the eIF3a/TIF32 subunit and sharply declines with the extended length of uORFs. Our data thus imply that eIF3 travels with early elongating ribosomes and that the RPEs interact with eIF3 in order to stabilize the mRNA-eIF3-40S post-termination complex to stimulate efficient reinitiation downstream.

Keywords: *Translational control, Reinitiation, uORF, eIF3, RNA-protein binding assay*

[PS10-10] Mutations outside of Ure2 amyloid-forming region disrupt [URE3] prion propagation by different mechanisms.

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Ure2 protein is a key transcriptional regulator of nitrogen catabolism and the determinant of the yeast prion [URE3]. Its unstructured N-terminal domain (NTD) can misfold to form amyloid fibers that are the basis of the [URE3] prion. The globular C-terminal domain (CTD) performs the transcriptional regulation function.

To be inherited stably in dividing cells amyloid-based yeast prions must replicate so their numbers keep pace with the expanding population. Replication depends on fragmentation of prion fibers by the cytosolic protein disaggregation machinery, which is driven by Hsp104 and requires assistance by Hsp70 and its Hsp40 and nucleotide exchange factor co-chaperones. The normal disaggregating activity of this machinery extracts monomers from the prion polymers, which causes them to break.

In contrast, altered abundance or activity of any of these chaperones, or several other protein quality control (PQC) factors, can cure yeast of prions. Also, endogenous cellular activities of some PQC factors have anti-prion functions. At normal levels Btn2, Cur1 and Hsp42 prevent establishment and propagation of [URE3] prions by collecting disperse prion fibers into one or a few large aggregates, and Hsp104 interferes with establishment of many variants of [PSI+] prions.

While the regions of several yeast prion proteins that confer ability to form amyloid are clearly defined, our understanding of where and how factors act on prion polymers to facilitate their propagation or elimination is limited. To gain insight into these processes we identified mutations in Ure2 outside the amyloid-forming region that disrupt ability of Ure2 to propagate as [URE3], reasoning that they might locate sites of interaction for chaperones or other factors rather than affecting amyloid propagation directly. Indeed, inhibition of [URE3] by several CTD mutations depended on expression levels of PQC factors.

Intriguingly, although all the mutant proteins possess wild type amyloid-forming regions, several CTD mutations altered the ability of Ure2 to form amyloid in vitro, which implies that disperse sites outside the amyloid-forming region are involved in interactions important for formation of amyloid.

Keywords: *heat shock proteins, protein chaperones, Ure2, prion, anti-prion systems*

[PS10-11] Calmodulin Regulates Protease versus Co-chaperone Activity of Metacaspase - Turning an Executioner into a Protector

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Metacaspases are ancestral homologues of caspases that can either trigger apoptosis upon cytotoxic stress or protect cells against proteotoxic stress and slow cellular aging. We show that the yeast metacaspase Mca1 display dual activities; canonical metacaspase protease activity and co-chaperone-like activity. The former activity was inhibited by calmodulin binding to the N-terminal domain of Mca1. Mca1 lacking this domain displayed constitutive activation and elevated protease activity and could no longer extend lifespan when overproduced. Moreover, in conditional cmd1 calmodulin mutant cells, Mca1 became auto-catalytically activated and triggered apoptosis. Calmodulin, however, did not inhibit the ability of Mca1 to boost Hsp70- dependent protein refolding, suggesting that Cmd1, by binding to the N-terminal domain of Mca1, favor co-chaperone over protease activity and that this regulation of Mca1 is key to the protein being executioner or protector.

Keywords: *Metacaspase, aging, apoptosis, protein quality control*

[PS10-12] Analysis of spatial segregation of the eIF3a mutant in Saccharomyces cerevisiae

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Yeast Saccharomyces cerevisiae has elaborated a strategy by which potentially harmful protein aggregates are retained within the mother cell and the pristine daughter cells have the full replicative potential. This process is called rejuvenation and it is a typical example of spatial protein quality control (SQC).

We present here an analysis of a mutant form of the translation initiation factor eIF3a/Rpg1 that is an aggregate-prone since it forms foci already at physiological growth conditions. There are two major types of foci observable, small ones that are mobile and reach the daughter cell cytosol, and large ones that display a restricted mobility and are retained within the mother cell in the proximity of the nucleus. The ability of small accumulations to escape from SQC of the mother cell is intriguing because it resembles a prion-like behavior, however, they do not form amyloids. A microscopy analysis of the movement of small GFP-labeled foci after application of 2-deoxyglucose and FCCP when the foci became immobile

lead to a conclusion that their movement is active and dependent on energy. A trajectory analysis of the foci motility clearly showed that the foci movement is directed mostly from mother to daughter cell. It seems that intact microtubules are dispensable for the movement of foci since their depolymerization by nocodazole affected neither the foci trajectory nor the velocity. However, depolymerization of actin cables by Latrunculin B interrupted the movement of foci to daughter cells. Surprisingly, we did not observe any changes in the mobility of foci under the absence of the actin-based motor Myo4 and the protein adaptor She3. It seems thus that other transport molecules dependent on intact actin cables might carry mutant Rpg1 foci into daughter cells.

This work was supported by the grant from the Czech Science Foundation CSF16-05497S.

Keywords: *spatial protein quality control (SQC), prion-like behavior, trajectory analysis*

[PS10-13] Study of the Function of Nst1 as a Component of the Stress-induced mRNP Granules in Saccharomyces cerevisiae

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Life is stressful. The question of how cells respond to stressful environments for survival is critical to understand life. In stress conditions such as glucose depletion, oxidative stress, osmotic stress etc., mRNA and Protein complexes (mRNP) are known to accumulate rapidly and assemble into mRNP granules for translational regulation. mRNP formation is a well-established phenomenon highly conserved in all eukaryotic cells. The most representative mRNP granules are P-bodies and Stress Granules, containing mRNA decay proteins and mRNA translation initiation complex, respectively. P-bodies and Stress Granules are assembled in the presence of stress, transformed to one another, and disassembled when the stress is alleviated. However the mechanisms of assembly, disassembly, and transformation of mRNP granules are still elusive. Here, we observed that Nst1, previously reported as an adaptor-like protein that assembles different MAP kinase modules allowing the cross-talk between MAP kinase pathways, was also localized in both P-bodies and Stress Granules in glucose-depleted stationary phase cells. Furthermore P-body marker proteins were strongly accumulated in Nst1 overexpressed cells. These results indicate that Nst1 may have roles in mRNP granule assembly in response to stress.

Keywords: *mRNP Granule, Nst1, Stress-response*

[PS11] Yeast prions and heat shock proteins

[PS11-1] Evolutionary Adaptation to a Foreign Hsp90 in the Budding Yeast Saccharomyces cerevisiae

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Heat shock protein 90 (Hsp90) is a molecular chaperone essential for cell viability in all eukaryotic cells. In yeast cells, the whole proteome is influenced profoundly when the Hsp90 activity is reduced. Despite the pivotal role that Hsp90 plays in life, our orthologue replacement assays revealed incompatibility between the Yarrowia lipolytica (Yl) HSP90 and the Saccharomyces cerevisiae genome, suggesting that its interaction network has changed through evolution. To understand how Hsp90 diverged in different species and the underlying driving force, we evolved the Yl-HSP90-carrying S. cerevisiae strain in multiple independent lineages in the normal growth condition (YPD 28°C). After 2,200 generations of evolution, we found that other than improving fitness in the normal condition, different evolved clones gained additional growth advantages in various stress conditions, indicating that they have adapted through different evolutionary trajectories. We sequenced the evolved clones and found that many mutations occurred in Hsp90 clients or client-related proteins. Moreover, several genes involved in protein homeostasis were mutated, suggesting that cells could relieve the Hsp90 defects by enhancing alternative pathways. Bulked segregate analysis was performed in three evolved clones to identify the mutations that have major contributions. In total we identified 10 mutations and confirmed the effect by reconstituting them in ancestral strains. Our results provide the potential explanation for the divergent evolution of Hsp90-interaction network and demonstrate the possible compensatory mutational paths that cells could apply when an essential network is perturbed.

Keywords: *Hsp90, experimental evolution, adaptive mutation*

[PS11-2] Mechanisms of Prion-Dependent Lethality In Yeast

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Prions are self-perpetuating protein conformations which often have an amyloid nature. In yeast amyloid prions manifest as heritable traits as their presence affects normal function of prion proteins. E.g., [PSI+], the prion form of translation termination factor Sup35 (eRF3), decreases accuracy of stop codon recognition and exhibits nonsense-suppressor phenotype.

Additional production of Sup35 in [PSI+], but not [psi-] cells, is toxic. This phenomenon is referred to as prion-dependent lethality or prion toxicity.

We screened for Q/N-rich transcription factors affecting [PSI+] propagation and found that excess of several factors enhanced [PSI+]-dependent lethality. The lethality was accompanied by elevation in SUP35 mRNA levels in [PSI+] strain, suggesting transcriptional upregulation as the mechanism. One of the factors, Sfp1, was shown not only to upregulate SUP35, but also SUP45, while additional expression of SUP45 is known to alleviate the [PSI+] toxicity. We showed that, indeed, SUP45 overexpression compensated [PSI+]-dependent lethality caused by excess Sup35, but did not affect Sfp1-derived lethality, implying involvement of another mechanism. Search for the factors that influenced the lethality of excess Sfp1 led to identification of Hsp40 chaperone Sis1 which alleviated toxicity of excess of both Sfp1 and Sup35 in [PSI+] strains. Sfp1 colocalized with [PSI+] aggregates and its overproduction also affected Sup35 aggregate size, while coexpression of Sis1 returned the size distribution to normal. Thus at least two different mechanisms are involved in [PSI+]-dependent lethality.

Sis1 is known to counter the toxicity of many prions and amyloids in yeast, such as Rnq1/[PIN+] and [PIN+]-dependent polyQ-toxicity, but the underlying mechanisms are not entirely understood. Recently we showed that partitioning of Sis1 between cellular compartments may affect different prions in different ways. [PSI+] is less sensitive to alterations is Sis1 than other prions. Relocalization of most cellular Sis1 into the nucleus causes [PSI+]-dependent growth defect, accompanied by enhancement of [PSI+], suggesting that cytoplasmic Sis1 normally aids in detoxifying [PSI+] aggregates, allowing non-lethal [PSI+] propagation.

The work was supported by RC MCT SPbSU and by grants from Saint-Petersburg State University (15.61.2218.2013; 1.37.291.2015), RFBR (16-04-00202; 15-04-08159), and by the grants of the President of the Russian Federation (MK-512.2017.4; NS-9513.2016.4).

Keywords: *yeast prions, prion toxicity, [PSI+], regulation of transcription, Sfp1, Sis1*

[PS11-3] Spatiotemporal Distribution and Balance of Functional Cur1 and Sis1 Proteins Inversely Affect [PSI+] and [URE3] Propagation

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Yeast self-perpetuating protein aggregates (prions) propagate via tight interaction with molecular chaperones and protein sorting factors. Previously, we showed that overproduction of the chaperone sorting factor Cur1 oppositely affects yeast prions, curing [URE3] and [NRP1C+] while enhancing [PSI+]. The deletion analysis of the CUR1 gene showed that C-terminal region (31-252 aa) of the protein is critical for its effects on prions. Deletions of N-terminal regions (3-22 and 3-30) lead to opposite effects: Δ3-22 (has intact NLS) enhances pro-[PSI+] and anti-[URE3] effects, while Δ3-30 (lacking NLS) significantly reduces them. We link these effects to the ability of Cur1 to relocalize the Hsp40-Sis1 from cytoplasm into the nucleus as we demonstrated that both curing of [URE3] and enhancement of [PSI+] in the presence of excess Cur1 or Cur1Δ3-22 are counteracted by Sis1 in a dosage-dependent manner. Importantly, variant of Sis1, lacking its dimerization domain (Sis1ΔDD), is significantly less efficient in compensating effects of Cur1 on prions. Our findings emphasize the importance of spatiotemporal balance of functional Cur1 and Sis1 proteins for prion propagation.

Equipment of the Resource Center “Development of Molecular and Cell Technologies” at Research Park of St. Petersburg State University was used in this study. The study was supported by St. Petersburg State University (projects 15.61.2218.2013 and 1.37.291.2015) and grants from Russian Foundation for Basic Research (16-04-00202 and 15-04-08159), Russian Science Foundation (14-50-00069) and National Science Foundation (MCB 1516872).

Keywords: *yeast prion, Cur1, Sis1, [PSI+], [URE3], chaperones*

[PS11-4] Prion-like Properties of a Yeast G Protein Receptor Involved in Regulation of Mating

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G-protein-coupled receptors (GPCRs) are integral membrane proteins that initiate responses to extracellular stimuli by mediating ligand-dependent activation of cognate heterotrimeric G proteins. Ste18 is a gamma-subunit of a G-protein receptor that is conserved in evolution and plays a key role in a variety of cellular processes, including pheromone-signaling pathway that is crucial for the yeast mating. We demonstrate that Ste18 possess prion-like properties. Upon overproduction, Ste18 forms detergent-resistant amyloid-like aggregates and promotes formation of [PSI+], a prion isoform of another protein, Sup35/eRF3. Ste18 mutants, defective in anchoring to plasma membrane, are not able to form detergent-resistant aggregates or induce [PSI+] prion, while a mutant, deficient in signal transduction but not in membrane anchoring, is able to do so. These data show that prion-like properties of Ste18 depend on its association with a membrane and resemble our previous results for another protein, Lsb2 (see Chernova et al., 2017 Cell Reports 18: 751-761), whose prion properties depend on association with a peripheral actin cytoskeleton. Overall, our findings emphasize the significance of a specific intracellular location for prion formation. Similar

to Lsb2, Ste18 is ubiquitinated, short-lived and degraded by a proteasome. Levels of Ste18 protein are increased in the conditions when proteasome function is impaired, suggesting that aggregate formation by Ste18 may occur in response to the malfunctioning or overload of the ubiquitin-proteasome system, for example during a proteotoxic stress. Potential involvement of prion-like aggregation in regulation of G-protein dependent signaling and yeast mating will be discussed in the light of both our data and recent developments, suggesting the role of protein aggregation not only in diseases, but also in regulation of some biological processes.

Keywords: *G-protein, prion, amyloid, proteasome, aggregation, ubiquitin*

[PS11-5] Multiple Stress-Protection Functions of DJ-1/ThiJ/PfpI Family Proteins: Lessons from Saccharomyces cerevisiae

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Saccharomyces cerevisiae Hsp31p protein belongs to the ubiquitous DJ-1/ThiJ/PfpI family. Its most prominent member is human DJ-1 whose defects are being associated with Parkinson's disease pathogenesis. No wonder that its budding yeast relative attracts so much attention. Numerous recent findings reported by us and others, revealed its importance for survival in the post-diauxic phase of cell growth and under diverse environmental stresses. It was shown to posses glutathione-independent glyoxalase III activity and also a function of protein chaperone, which suggests it has multiple cellular roles. Accordingly, our previous study revealed also that HSP31 gene expression is controlled by multiple stress-related transcription factors. They mediate the HSP31 promoter responses to oxidative, osmotic and thermal stresses, to toxic products of glycolysis, such as methylglyoxal and acetic acid, and to the diauxic shift. Since Hsp31p glyoxalase III coexists in S. cerevisiae cells with thousand-fold more potent glyoxalase I/II system, its biological purpose requires substantiation. Important contribution that may reveal the true role of this protein in budding yeast comes from our findings on subcellular localization of this protein. We demonstrated that, unlike glyoxalase I/II system enzymes, which are localized in the cytosol, Hsp31p protein is present in the periplasmic space. This localization is compatible with its postulated chaperone activity, since other known chaperone proteins of Hsp70 family are also present in this compartment. Yet it is also consistent with its glyoxalase III activity being first line of defense against exogenous methylglyoxal present at high concentrations in some natural environmental niches populated by budding yeast.

Our findings make budding yeast not so perfect model in Parkinson's disease studies since human DJ-1, Hsp31p ortholog, is found inside the cell: in the cytosol and in mitochondria, though its postulated activities are also diverse. Nevertheless, periplasmic localization of Hsp31p brings S. cerevisiae model closer to less distant, yet no less medically important relative, Candida albicans. In this pathogen, DJ-1/ThiJ/PfpI family protein called Glx3, orthologous to ScHsp31p, also has glyoxalase III activity. Genome-wide proteome data

suggest its periplasmic localization and the potential role in virulence, perhaps as protectant against methylglyoxal secreted by host immune cells to combat microbial infections.

Keywords: *Candida albicans, periplasm, methylglyoxal, model organism, multifunctional, chaperone*

[PS11-6] A New Collection of [PSI] Strains

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Twenty-two different prion strains of the wild-type Sup35 protein were collected, including 18 novel ones in addition to 4 previously reported. Prion particles were prepared from the cell to infect prion-less yeast and impart characteristic phenotypes. Infectivity was further generated de novo by nucleating bacterially prepared Sup35 protein with the yeast particles. Sup35 strains independently isolated by other laboratories were identified within the collection. The well-characterized 22 prion strains offer a good starting point to investigate how a single protein sequence folds into so a great many structures.

Keywords: *yeast prion, prion strain, amyloid, Sup35, [PSI]*

[PS11-7] Seeding of in Vitro Amyloid Aggregation by Endogenous and Artificially Produced Aggregates from Yeast Cells

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Protein Misfolding Cycling Amplification (PMCA) is a cutting-edge technique for seeding aggregation of prions and other amyloidogenic proteins in vitro by aggregates produced in vivo. This technique can be applied to the detection of prions and other amyloids in biological samples, however successful application of PMCA needs careful adjustment of experimental protocol for each new protein. Here we develop the PMCA protocol for seeding amyloid aggregation of yeast Sup35NM (truncated form of the Sup35 prion protein) by lysates of yeast cells containing [PSI+], a prion form of the Sup35 protein. We compared efficiencies of seeding in quiescent conditions, where the monomeric form of Sup35NM did not show any sign of spontaneous aggregation during the period of experiment, and in shaking conditions, where spontaneous aggregation does occur. Our data indicate that the lag period of aggregation reaction inversely correlates with the amount of the “seed”, added to the reaction mixture, thus allowing to detect presence of Sup35 amyloids in the extracts of yeast cells by PMCA. The major obstacle we have encountered in our PMCA experiments was associated with the high proteolytic activity of yeast extracts, leading to degradation of

the substrate Sup35NM protein. Approaches used to minimize the impact of proteolytic activity will be discussed. Finally, we extend the PMCA technique to detection of amyloids, formed by the chimeric constructs containing human amyloid beta (Abeta) peptide (associated with Alzheimer disease) in the yeast cells. The yeast-based protocol can be employed to optimize conditions for the PMCA-based detection of Abeta polymers in human samples.

Keywords: *Saccharomyces cerevisiae, Sup35NM, PMCA*

[PS11-8] Mapping the amyloid cores of [PSI+] prion variants

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The [PSI+] prion related to the Sup35 (eRF3) protein shows phenotypic variation, with variants differing by the strength of nonsense suppression and mitotic stability. The number of possible variants is unclear: there could be just two, or very many. The structural basis of this variation is only partly characterized. To understand better the structure of [PSI+] variants, we mapped the proteinase K -resistant cores of Sup35 prion particles, isolated from yeast cells of about 20 [PSI+] isolates with varying properties. In all preparations, the first 31 residues were fully protected and the first 70 were protected partly. We consider this 70-residue region as a proximal prion core. Two distinct digestion patterns were observed for this region, which correlated with the "strong" and "weak" [PSI+] phenotypes. The proteinase-resistant peptides were also found in four other regions (distal cores) within Sup35 N and M domains. Protection of these regions differed between [PSI+] isolates. It appears that the distal cores do not significantly affect the [PSI+] phenotype.

The work was supported by the Russian Scientific Foundation grant 17-14-01092.

Keywords: *prion, prion variant, prion structure, [PSI+], Sup35, proteinase K, MALDI*

[PS11-9] HSP12 as a Common Goods Cooperation and Programmed Cell Death at the Community Level

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Programmed cell death and apoptosis in yeasts make sense in a theoretical framework that goes beyond the biology of the single strain, but extends organismal behaviors to cells of different strains from the same species, the “clan”. The ecological life cycle of Saccharomyces cerevisiae assumes the prevalence of Cerevisiae strains by production of toxins such as ethanol and acetic acid, in sugar rich environment. Yet the coexistence of different yeasts in a single environment raises the question on how they communicate and why slow growers or apparently “unfit” strains, are not competed out. Aim of our work is to

investigate ecological interactions of “natural” strains, with different life span and different levels of Ethanol production, acetic acid production, and production of quorum sensing molecules, in YPD, SWM, YPL and ecologically relevant settings such as the wasps gut. Following the assessment of differential fitness in the different growth conditions, we investigate the genes regulating cooperation and competition. We present evidences of Hsp12p-dependent altruistic and contact-dependent competitive interactions between different natural yeast isolates. Hsp12p is released during cell death for public benefit by a fast-growing strain that also produces a killer toxin to inhibit growth of a slow grower that can enjoy the benefits of released Hsp12p. We also show that the protein Pau5p is essential in the defense against the killer effect. Finally we show that quorum sensing molecules regulate the timing of this phenomenon, thus suggesting active mechanism for controlling the utilization of dead cells in the co-culture, through a combination of policing and cooperative mechanisms, determining community level fitness in different ecological settings.

Keywords: *yeast sociobiology, cell death and apoptosis, yeast systems biology, evolutionary biology*

[PS11-10] Polyprionic Inheritance

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We have shown that manifestation of yeast prion factor [NSI+] (Nonsense Suppression Inducer) is controlled by interaction of two prions [1]. [NSI+] has pleotropic manifestation and in particular was shown to suppress the ade1-14UGA nonsense mutation at the background of modified SUP35 variants with deleted N-terminal prion-forming domain [2; 3]. [NSI+] demonstrates all characteristics of yeast prions: reversible curability, dominant non-Mendelian inheritance, and cytoplasmic infectivity. Deletion of the chaperone Hsp104 or its inactivation by guanidine hydrochloride causes elimination of [NSI+]. To identify protein determinants of [NSI+] we used our original method of proteomic screening for prions named PSIA-LC-MALDI. This method is based on the resistance of prion aggregates to treatment with ionic detergents such as sodium dodecyl sulfate (SDS). Using this method we showed that [NSI+] strain, in contrast to [nsi-], contains the Swi1 and Rnq1 proteins in prion forms [SWI+] and [PIN+], correspondingly. Prion inactivation of Swi1 causes a decrease in SUP45 expression that leads to the weak suppressor phenotype. [PIN+] does not cause nonsense suppression itself, but strongly enhances the effect of [SWI+] [1]. These data show that two prions, like classical genes, demonstrate complementary interactions, and this interaction causes heritable changes in yeast cells. Thus, by analogy with monogenic and polygenic inheritance, in the framework of the prion concept we can distinguish “monoprionic” and “polyprionic” types of inheritance.

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Keywords: *prions, interaction, inheritance, epigenetics*

[PS11-11] Novel functional amyloids in yeast identified by proteomic approach

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Amyloids are protein fibrils that are stabilized by intermolecular beta-sheets that are stacked in the direction perpendicular to the fibril axis. Functional and pathological amyloids are presented in a broad range of organisms from bacteria to mammals. Some amyloids called “prions” are infectious and may be transferred from one organism to another. The infectivity of prion fibrils is determined by their ability for reproducible cycles of replication. Despite extensive study, our knowledge about amyloids is very fragmentary. We cannot estimate how amyloids are widespread, because all standard approaches are limited to the analysis of proteins similar to known amyloids or searching for determinants of known pathologies or phenotypic changes. Recently we developed and successfully validated a method of proteomic screening for amyloids, which is based on the resistance of amyloid fibrils to treatment with ionic detergents such as sodium dodecyl sulfate (SDS) [1; 2]. We applied this method to identify the proteins which form amyloid-like detergent-resistant aggregates in the proteome of yeast strains that have different origin. Based on the result of proteomic screening we compiled a list of proteins that are candidates for the role of functional amyloids. The list of proteins forming SDS-resistant aggregates includes cell wall amyloid Bgl2, well-known yeast prions, and set of the proteins whose amyloid properties have not previously been shown. In particular, we identified several RNA-binding proteins and cell wall proteins such as Gas1, Gas3, Gas5, Toh1 and Ygp1. Two proteins from this list were selected for detailed analysis of amyloid properties. We confirmed that Gas1 and Ygp1 proteins demonstrate amyloid properties in yeast cells and generate extracellular amyloid fibrils in vivo in bacteria-based system called curli-dependent amyloid generator [3]. Our approach opens the opportunity for characterizing amyloid landscape in proteomes of different organisms that is important for understanding of the role of amyloids in the regulation of cellular processes. This work was supported by grant from Russian Science Foundation (Project 14-50-00069). Authors acknowledge the SPbSU Resource Centers «CHROMAS» and «Molecular and Cell Technologies» for technical support.

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Keywords: *prions, functional amyloids, proteomic screening*

[PS12] Yeast as a model for human diseases and drug testing

[PS12-1] Proteostasis Impairment and Endoplasmic Reticulum Stress in a Yeast Model for Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis is a neurodegenerative disease that affects motor neurons. The majority of ALS cases (90%) are sporadic. More than thirty genes have been associated with familial cases so far. The P56S mutation in the protein VAPB was associated with ALS8 in Brazilian patients. VAPB is a membrane protein of the endoplasmic reticulum that is possibly involved in diverse cellular functions, including intracellular trafficking, interaction endoplasmic reticulum-Golgi and Unfolded Protein Response. Here, we aimed to analyze the endoplasmic reticulum stress and protein degradation pathways as factors underlying the pathogenicity of VAPB. The BY4741 strain of the yeast Saccharomyces cerevisiae expressing VAPBWT or VAPBP56S was employed as a model. Initially, it was verified that both VAPBWT and VAPBP56S were located in the endoplasmic reticulum, and the VAPBP56S protein was found as SDS-resistant aggregates. Expression of the VAPBP56S but not of VAPBWT was toxic, reducing cell viability. Since proteasome and autophagy are two processes that protect cell against toxicity associated with aggregates, the effects of their inhibition were investigated. Proteasome was inhibited by the use of the MG 132 compound (under the Δpdr5 background), reducing the viability of strains expressing VAPBWT and the rate of VAPBWT degradation. In contrast, autophagy inhibition (achieved by atg8 gene deletion) affected mainly the viability of cells expressing VAPBP56S. Cells expressing VAPBWT and VAPBP56S displayed increased levels of proteasome subunits. As expected, in cells expressing VAPBWT the proteasome activity was increased and the pool of ubiquitilated proteins was diminished. Surprisingly, in cells expressing VAPBP56S proteasome activity was reduced and ubiquitilated proteins accumulated at much higher levels. The autophagosomes formation (assessed by GFP-Atg8 fusion) was increased only in strains expressing VAPBP56S, suggesting higher levels of autophagy. Finally, the levels of endoplasmic reticulum stress markers (pdi1, ero1, lhs1 and kar2) were induced in cells expressing VAPBP56S. Taken together, our data suggest a link between proteostasis, endoplasmic reticulum stress and VAPBP56S in our ALS model, with autophagy playing a predominant role in the protection against toxicity associated with aggregates formation.

Keywords: *Amyotrophic Lateral Sclerosis, Proteostasis, Endoplasmic Reticulum Stress, Disease Model, Proteasome, Autophagy*

[PS12-2] Amino Acid Substitution Equivalent to Human Chorea-acanthocytosis I2771R in Yeast Vps13 Protein Affects its Binding to Phosphatidylinositol 3-phosphate

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The rare human disorder chorea-acanthocytosis (ChAc) is caused by mutations in hVPS13A gene. The hVps13A protein interacts with actin and regulates the level of phosphatidylinositol 4-phosphate (PI4P) in membranes of neuronal cells. Yeast Vps13 is involved in vacuolar protein transport and, like hVps13A, participates in PI4P metabolism. Vps13 proteins are conserved in eukaryotes, but their molecular function remains unknown. One of the mutations found in ChAc patients causes amino acids substitution I2771R which affects the localization of hVps13A in skeletal muscles. To dissect the mechanism of pathogenesis of I2771R, we created and analyzed a yeast strain carrying the equivalent mutation. Here we show that in yeast, substitution I2749R causes dysfunction of Vps13 protein in endocytosis and vacuolar transport, although the level of the protein is not affected, suggesting loss of function. We also show that Vps13, like hVps13A, influences actin cytoskeleton organization and binds actin in immunoprecipitation experiments. Vps13-I2749R binds actin, but does not function in the actin cytoskeleton organization. Moreover, we show that Vps13 binds phospholipids, especially phosphatidylinositol 3-phosphate (PI3P), via its SHR_BD and APT1 domains. Substitution I2749R attenuates this ability. Finally, the localization of Vps13-GFP is altered when cellular levels of PI3P are decreased indicating its trafficking within the endosomal membrane system. These results suggest that PI3P regulates the functioning of Vps13, both in protein trafficking and actin cytoskeleton organization. Attenuation of PI3P-binding ability in the mutant hVps13A protein may be one of the reasons for its mislocalization and disrupted function in cells of patients suffering from ChAc.

Keywords: *Vps13, chorea-acanthocytosis, yeast model, actin cytoskeleton, binding of lipids, protein trafficking*

[PS12-3] A Yeast-based Approach to Assess Mechanisms of Toxicity of Natural and Synthetic Naphthoquinones

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The quinone moiety is frequently found in natural products obtained from plants and microorganisms. Many of these products possess strong antimicrobial and antiparasitic activities and some have been introduced in the clinic to treat diseases such as cancer and

malaria. Quinones may exert their biological activities through three basic mechanisms: (i) reactivity against cell nucleophiles; (ii) one or two electron redox cycling in the presence of dioxygen; and (iii) inhibition of key enzymes or structural proteins such as topoisomerase II, protein tyrosine phosphatases, tubulin, etc.

The yeast Saccharomyces cerevisiae is particularly suited to study chemical-biological interactions of quinones because of its unique ability to perform aerobic fermentation and grow in the absence dioxygen and mitochondria [1]. We have used these special features to characterize the mechanism of toxicity of several natural and synthetic naphthoquinones related to lawsone, juglone and lapachol. We have found we can classify yeast cytotoxic naphthoquinones in three groups in terms of mechanism of toxicity. The first group comprises quinones whose toxicity greatly depends on dioxygen, exert also a strong oxidative stress to the cell, and selectively target mitochondrial function. The second group comprises quinones which shared with the first group the generation of oxidative stress, but fail to target mitochondria significantively. The third group includes quinones whose cytotoxicity goes beyond any involvement of dioxygen, oxidative stress or mitochondrial function. Surprisingly, even minor semisynthetic modifications in the products are able to change the mode of cytotoxicity for the studied quinones. A structure-activity relationship analysis will be presented.

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Keywords: *Chemical biology, Quinones, Oxidative stress*

[PS12-4] Saccharomyces cerevisiae as a Model to Study TIR-Domain Containing Proteins in Host-Pathogen Interactions

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Toll/Interleukin-1 Receptor (TIR) domains are protein-protein interaction folds present in supramolecular complexes involved in the detection of pathogen-associated molecular patterns (PAMPs) to trigger the innate immunity response in vertebrates. Thus, many intracellular pathogens have evolved to interfere with the assembly of these complexes. For example, Brucella spp. are able to down-regulate the host innate immune response by translocating the BtpA and BtpB Type 4 Secretion System (T4SS) effectors that mimic the

higher eukaryotic TIR domains in Toll-Like-Receptor (TLR) signaling pathway adaptor proteins. Our study aims to provide insights into the function of these bacterial virulence factors and to characterize TIR-TIR interactions using Saccharomyces cerevisiae as a model via heterologous expression.

Both BtpA and BtpB Brucella abortus TIR protein GFP fusions had a growth inhibitory effect upon expression in yeast, which was more severe in the case of BtpB. Fluorescence microscopy showed GFP-BtpA decorating yeast nuclei whereas GFP-BtpB displayed a punctated cytoplasmic pattern. GFP-BtpB expression led to depolarization of actin structures, a severe blockage of endocytosis and a general defect of kinase activity in S. cerevisiae signaling pathways. A pooled whole genome cDNA library (Yeast ORF collection, Dharmacon) screening for suppressors by overexpression led to 16 positive ORF that rescued BtpB toxicity in yeast, currently being assed for relevance. The C-terminal half of BtpB(140-292) bearing the TIR domain was responsible for all BtpB phenotypes in yeast, and random mutagenesis revealed that conserved residues in the TIR domain were essential for toxicity. Interestingly, GFP-BtpB(140-292) formed long and curved cytoplasmic structures in S. cerevisiae. We co-expressed four fluorescent protein fusions of human TLR signaling adaptor proteins: MyD88, TIRAP, TRIF and TRAM, all TIR domain-containing proteins, together with BtpA and BtpB. Both positive and negative genetic interactions, as well as subcellular co-localization phenomena were observed, suggesting that yeast is an excellent platform to test TIR-TIR interactions for function and specificity in vivo. We propose this tool for molecular studies on these molecular mechanisms, which are crucial for the evasion of the immune response characteristic of intracellular pathogens.

Keywords: *Heterologous expression, TIR domain, TLR signaling, Saccharomyces cerevisiae*

[PS12-5] RCN2 Encoding the Calcineurin Regulator Is a Suppressor of vps13 Mutations in Yeast Chorea-acanthocytosis Model

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Chorea-acanthocytosis (ChAc) is a fatal rare genetic neurodegenerative disease linked with mutations in hVPS13A gene, one of four VPS13 genes in human. Mutations in hVPS13B and hVPS13C are also implicated in human neurodegenerative disorders and effective cure for any of these diseases is lacking. VPS13 genes are conserved from yeast to humans. Thus, yeast is a good model system to study function of Vps13 proteins, the effect of human mutations on cell physiology and to screen for suppressors of mutations in VPS13 gene. In yeast there is one VPS13 gene and it is most homological to hVPS13A. The deletion of VPS13 gene in yeast impairs many functions such as intracellular trafficking, actin cytoskeleton organization and sporulation. A point mutation vps13I2749R, which mimics the point mutation found in ChAc patient, also exhibits lost of function phenotypes. We identified RCN2 gene as a multicopy suppressor of vps13Δ, as well as vps13I2749R mutation. RCN2, next to RCN1, is the regulator of calcineurin, a calcium and calmodulin dependent protein phosphatase. While RCN1, depending on the expression level and phosphorylation state, can stimulate and inhibit calcineurin, RCN2 shows only inhibitory activity if overexpressed.

Here we show that overexpression of RCN2 diminishes sensitivity to canavanine and improves actin cytoskeleton organization of vps13 mutant cells. Our results suggest possible link between calcium signaling and function of Vps13.

Keywords: *Yeast, Vps13, Chorea-acanthocytosis, Rcn2, Calcium signaling*

[PS12-6] Physiological and transcriptome analyses of amyloid-β peptide-induced cytotoxicity in Saccharomyces cerevisiae

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Alzheimer disease (AD) is the most common form of dementia, and it is a progressive, incurable and fatal disease. It affects approximately 36 million people worldwide, and will become the world's leading cause of death by 2050 due to increasing longevity. The key pathological hallmark of AD is the accumulation of insoluble plaques in the brain, which are preferentially composed of aggregated amyloid-β protein (Aβ). The exact mechanism of Aβ accumulation is poorly understood, and thus it is difficult to develop effective treatments. The yeast Saccharomyces cerevisiae shares many conserved biological processes with all eukaryotic cells and has become a valuable tool to unravel fundamental intracellular mechanisms underlying AD. We constructed a model for Aβ localization and toxicity by expressing and directing human Aβ peptides (Aβ40 and Aβ42) to the secretory system in yeast. The cells constitutively producing Aβ exhibited a reduced growth rate and shorter chronological life span. Additionally, the more toxic Aβ42 expression strain suffered from decreased mitochondrial function which was accompanied by an elevated production of reactive oxygen species (ROS) and ubiquitin-proteasome system dysfunction (1).

To better understand the physiology of Aβ-expressing strains, we took advantage of our humanized yeast model to further exploit the effects of Aβ on cellular functioning, viability and energetics following a systems biology approach. By controlling the culture parameters, we reduced the number of irrelevant and sidetracking variables and produced a considerable amount of genome-wide and physiological information concerning the energetic consequences of Aβ expression, as well as revealing how these different Aβ toxic isoforms interfered with cellular metabolism and stress response pathways, causing pronounced physiological effects.

Keywords: *Amyloid-β, Alzheimer's disease, Energetics, ER stress, yeast*

[PS12-7] Cancer-associated Isocitrate Dehydrogenase Mutations Induce Mitochondrial DNA Instability in Saccharomyces cerevisiae

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The identification of early-occurring mutations in the NADP+-dependent isocitrate dehydrogenase genes IDH1 and IDH2, is a major advance in understanding the progression and prognostic outcome of certain cancers, such as low-grade gliomas, acute myeloid leukemia, and chondrosarcomas. These mutations frequently occur at conserved arginine residues important for binding isocitrate, resulting in loss of the wild-type enzymatic activity and in a neomorphic activity, which overproduces the onco-metabolite D-2-hydroxyglutarate (2HG), thought to contribute to disease progression. However, the mechanisms by which 2HG accumulation is cytotoxic and contribute to tumorigenesis are still unclear (1). To better understand the mechanisms of 2HG pathophysiology, we developed a novel yeast model to elucidate the biological impact of the onco-metabolite 2HG. We introduced the analogous glioma-associated mutations into the NADP+ isocitrate dehydrogenase genes (IDP1, IDP2, IDP3) in S. cerevisiae. Intriguingly, expression of the mitochondrial IDP1R148H mutant allele results in high levels of 2HG production as well as extensive mtDNA loss and respiration defects. We find no evidence for a reactive oxygen-mediated mechanism mediating this mtDNA loss. Instead, we show that 2HG production perturbs the iron sensing mechanism as indicated by upregulation of the Aft1-controlled iron regulon and a concomitant increase in iron levels. Accordingly, iron chelation, or overexpression of a truncated AFT1 allele that dampens transcription of the iron regulon, suppresses the loss of respirative capacity. Additional suppressing factors include overexpression of the mitochondrial aldehyde dehydrogenase gene ALD5 or disruption of the retrograde response transcription factor RTG1. Elevated α–ketoglutarate levels also suppress 2HG-mediated respiration loss; consistent with a mechanism by which 2HG contributes to mtDNA loss by acting as a toxic α–ketoglutarate analog. Our results are of interest in light of recent studies underscoring an upregulation of iron trafficking in glioma cells (3), and suggest that this mechanism could potentially result in the mtDNA damage often observed in tumor cells. Our findings provide insight into the mechanisms that may contribute to 2HG oncogenicity in glioma and acute myeloid leukemia progression.

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Keywords: *cancer yeast model, oncogenic IDH mutations, mechanisms of 2HG-pathophysiology and tumorigenesis, mitochondrial DNA loss, Iron toxicity*

[PS12-8] Specialized Yeast Ribosomes to Develop Methods for Therapeutic Readthrough of Premature Termination Codons in Rare Disease

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Ribosomes execute codon directed mRNA translation into proteins. Mutations that alter sense mRNA codons into unscheduled stop codons are termed premature termination codons (PTCs), also called non-sense codons. A PTC triggers the nonsense-mediated mRNA decay pathway and production of a truncated, potentially deleterious protein. PTC mutations account for about 25% of the known 8000 genetic diseases and most of those fall into the class of rare diseases, i.e. are identified in less than 1% of the population.

In severe cases of PTC alleles on muscular dystrophy and cystic fibrosis, respectively, administration of high doses of aminoglycosides has achieved PTC readthrough by reducing accuracy of decoding in the A-site of the ribosome, albeit with severe side effects to the patients (1). We show here that the versatile yeast translation system can be used to identify methods for therapeutic readthrough of premature termination codons.

Our approach demonstrates that altering the functional availability of individual ribosomal proteins (2) or that of individual rRNA nucleotide modifications (3) generates specialized ribosomes, which show preferential translation of selected mRNAs, including PTC reporters, in a background of largely unaltered bulk translation. We performed a specialized yeast ribosome screen by employing a large collection of diploid yeast strains, each deficient in one or other copy of the set of ribosomal protein genes, generating distinct populations of altered “specialized” ribosomes. Comparative protein synthesis assays were used to assess translational readout of different, heterologous mRNA reporters, including PTC reporters.

We identified ribosomal protein rpL35/uL29 as specific target for therapeutic intervention and repair of the human LAMB3-PTC mRNA. PTC induced loss of Lamb3 protein causes severe blistering of the skin and rare skin disease JEB-H (Herlitz junctional epidermolysis bullosa), as the trimeric laminin 5 complex - linking epidermis and dermis - can’t be formed in the absence of Lamb3 protein. We have developed, optimized and validated a cellular keratinocyte assay to investigate the impact of small molecules binding to rpL35/ uL29 to form a specialized ribosome for LAMB3-PTC repair.

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Keywords: *Specialized Ribosomes, PTC readthrough, Rare disease*

[PS12-9] Genetic dissection of an evolutionary conserved pathway regulating protein degradation

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Timely elimination of misfolded and damaged proteins is essential to maintaining cellular protein homeostasis. A functional decline in the proteolytic capacity of a cell leads to the accumulation of highly toxic protein aggregates that underlies the development of protein misfolding diseases such as Alzheimer's and Huntington's disease. The ubiquitin proteasome system (UPS) is the main cellular pathway for protein degradation where proteins modified by specific ubiquitin (Ub) signals such as K48-linked polyUb are targeted for degradation by the proteasome. We recently discovered a new DUB family, named MINDY, in mammalian cells. Interestingly, two members of this family are conserved down to yeast, and all MINDY DUBs are highly selective at cleaving K48-linked polyUb.

To understand the cellular role of this uncharacterized family of DUBs, we used S. cerevisiae, a powerful tool in the study of pathways regulating protein quality control and homeostasis. Being selective at cleaving K48-linked polyubiquitin, we hypothesized that MINDY DUBs are negative regulators of protein degradation. Indeed, yeast strains lacking MINDY (miyΔ) exhibit increased proteolytic capacity as assessed by degradation of model substrates. Excitingly, our results reveal that miyΔ strains not only have an extended chronological LifeSpan (CLS) but also exhibit an improved replicative LifeSpan. A decline in the proteolytic capacity of a cell is known to result in the accumulation of toxic protein aggregates, one of the underlying causes of ageing and age-related proteinopathies. Indeed, while wild type yeast cells accumulate protein aggregates with age, aged miyΔ cells have hardly any detectable protein aggregates.

In summary, our exciting results reveal that MINDY DUBs are hitherto unstudied important regulators of proteostasis. Further, by studying MINDY DUBs in human cells, my work reveals that the mechanism of action is evolutionarily conserved. Importantly, our results reveal that MINDY DUBs maybe an attractive therapeutic target in neurodegeneration.

Keywords: *Ubiquitin proteasome system, Deubiquitinase, protein misfolding, Proteinopathy*

[PS12-10] Uncovering Las17 secret service in nucleolus

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The yeast orthologue of the Wiskott-Aldrich Syndrome protein, Las17, is a key regulator of cortical actin polymerization. Disruption of LAS17 leads to the loss of actin patches and to block in endocytosis. Unexpectedly, when we used ASPIC-MS methodology to identify proteins bound to yeast chromosome XII containing rDNA repeats, aberrantly migrating on PFGE as chromosome cloud, we detected Las17. Using Las17-GFP fusion protein and the nucleolar marker Nop1-DsRed we confirmed the nucleolar localization of Las17. Further experiments comparing las17Δ mutant and the wild type strains revealed differences in nucleolar integrity, shape and positioning during cell division, suggesting a role of Las17 in nucleolar organization and division. The las17Δ cells showed also higher than wild type cells nucleolar fragmentation score after nocodazole treatment. Because Las17 overexpression protects cells against various stresses that affect the nucleolus, such as starvation, cell wall stress or oxidative stress, we postulate that the involvement of Las17 in cell growth regulation, extrachromosomal DNA transportation and stress responses is

linked to its role in nucleolar organization. We believe that newly assigned role of Las17 as a nucleolar protein engaged in nucleolar function, most likely nucleolar division, and ensuring nucleolar integrity might help to elucidate the etiology of the human immunodeficiency Wiskott-Aldrich Syndrome.

This work was supported by Polish National Science Center grant 2016/21/B/NZ3/03641

Keywords: *Las17, Saccharomyces cerevisiae, Wiskott-Aldrich Syndrome, nucleolus, aberrant DNA structure, DNA-binding protein*

[PS12-11] 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. Furthermore, linking somatic variants to specific cancer hallmarks, such as chromosome instability (CIN), is a major challenge in the cancer field. The budding yeast, Saccharomyces cerevisiae, has been utilized to define cellular pathways and catalog a comprehensive list of yeast genes required for the maintenance of chromosome stability (yeast CIN genes). Human orthologs of these yeast genes are candidate CIN genes whose tumor-specific variants may contribute to chromosome instability and tumorigenesis. By 'humanizing' a yeast strain using cross-species complementation, yeast can facilitate direct screening of these somatic mutations with the dual benefit that the variants are characterized in the context of the human gene and screened rapidly in a model eukaryote. Here, we outline an experimental approach to identify a list of human genes that can replace their yeast orthologs and complement a loss-of-function phenotype. We report the screening of essential and non-essential yeast CIN deletion mutants for complementation by their human counterparts. Replaceability was scored by examining rescue of growth defects caused by deletion of the essential yeast gene or by assaying for rescue of drug sensitivities/CIN defects for non-essential yeast genes. The resultant list of human gene/ yeast mutant complementation pairs has been used to test the functional consequences of cancer somatic mutations directly in yeast, while also providing a list of complementing genes as a resource to the field.

Keywords: *Chromosome instability, Human-yeast complementation, Tumor-specific variants*

[PS12-12] Deciphering Biochemical and Biophysical Properties of UPF0016 Membrane Proteins – A Link to Human Congenital Disorders of Glycosylation.

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Congenital Disorders of Glycosylation (CDG) comprise a group of rare inborn human diseases caused by defects in protein glycosylation. Recently, a subtype of CDG has been associated with mutations within the human protein TMEM165. This protein belongs to a family of poorly characterized membrane proteins (UPF0016) which is highly conserved through evolution and widely distributed among kingdoms. Recent results indicate that the UPF0016 proteins play a role in calcium, manganese and pH homeostasis. We have shown the S. cerevisiae UPF0016 member, Gdt1p, to be localized at the Golgi membrane, and to act as calcium and manganese transporter. Bioinformatic analysis of the UPF0016 family predicts three structural states formed through evolution: i) single-domain proteins with 3 transmembrane spans (TMD) which form homodimers, ii) single-domain proteins with 3 TMD (encoded by two adjacent genes on the chromosome) which form heterodimers, or iii) two-domains proteins with 6 TMD. To gain better insight into the conformational topologies of the three evolution states we selected seven genes from different UPF0016 subfamilies: TMEM165 (human), Gdt1 (S. cerevisiae), and five prokaryotic members, Ter1a and Ter2b (Trichodesmium erythraeum), which are predicted to form heterodimers, Dma (Desulfovibrio magneticus), which is predicted to form homodimers, and two (hyper)thermophilic genes from the archea Thermococcus gammatolerans and Pyrococcus furiosus. The codon-optimized synthetic genes were expressed in E. coli and L. lactis. By in vivo transport assays using the fluorescent dye Fura-2 we studied the calcium and manganese transport activity. Furthermore, purification of the proteins is being optimized for reconstitution into liposomes and in vitro transport assays. Altogether, a better understanding of the enzymatic activity, physiological role and structural aspects of the UPF0016 members will enable a better comprehension of the causal link between the development of CDG and the presence of mutations in the human gene TMEM165.

Keywords: *Membrane transporters, Calcium, Manganese, Golgi, UPF0016, GDT1, TMEM165*

[PS12-13] Oxidative Stress and Aging: Learning from Yeast Lessons

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Although aging is likely to be a multifactorial process, several pieces of evidence show that oxidative stress is connected to life span. Many questions remain unanswered: oxidative stress does indeed contribute to aging; are reactive oxygen species (ROS) only destructive agents or regulators of stress response and aging; is it the absolute level of oxidative stress or the response to oxidative stress, or a combination of both, that determines lifespan? Interest in the factors that determine longevity has increased since the life expectancy has increased and the world leading causes of death are age-related diseases, such as cancer and neurodegenerative diseases. The use of the yeast Saccharomyces cerevisiae as an experimental model in biochemical studies has enabled the understanding of basic cellular and molecular processes. Even taken into consideration the vast differences in complexity between yeast and humans, the study of oxidative stress response in yeast has provided key insights into the pathways that modulate human longevity. The entire genome sequence of yeast has been elucidated and it is amenable to genetic modifications, which facilitates the identification of drug targeting genes or stress response pathways. The reduced genetic

redundancy favors the visualization of the effect of the deleted or mutated gene. S.cerevisiae has similar antioxidant responses to mammals and over 25% of human degenerative disease related genes have close homologs in yeast. By manipulating growth conditions, yeast cells can survive only fermenting (low ROS levels) or respiring (increased ROS levels), which facilitates the elucidation of the mechanisms involved in the acquisition of tolerance to oxidative stress. Furthermore, the yeast databases are the most complete of all the eukaryotic models. In this work, we highlight the value of S. cerevisiae as a model to investigate the oxidative stress response and its potential impact on aging and age-related diseases, such as Alzheimer and Amyotrophic Lateral Sclerosis.

Keywords: *oxidative stress, aging, Alzheimer, ALS, Saccharomyces cerevisiae*

[PS12-14] Mitochondrial Diseases: Yeast as a Model for the Study of Suppressors

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Mitochondrial (mt) tRNA gene mutations are an important cause of human morbidity and are associated with different syndromes. Saccharomyces cerevisiae has been used as a model for studying the mt tRNA defects obtained by random or site-directed mutagenesis; by biolistic procedure we introduced in yeast genes human equivalent pathogenic mutations that impair the mitochondrial protein synthesis and OXPHOS ability.

We have previously shown that the mitochondrial protein synthesis elongation factor EF-Tu and isolated sequences from the carboxy-terminal domain of yeast and human mt leucyl-tRNA synthetases (LeuRS) have a wide range of suppression capability among different yeast mt tRNA mutants having defective respiratory phenotype. In the present study we report additional new results concerning the mechanism of suppression by the two mitochondrial protein synthesis factors and show that rescuing activity is closely related to the transcription level of suppressor. Additional, we show that the rescuing capability exerted by mt LeuRS can be restricted to a specific sequence of six amino acids from its carboxy-terminal domain. On the other hand by overexpressing a mutated version of mt EF-Tu in a yeast strain deleted for the endogenous nuclear gene we identified the specific region involved in suppression. Results support the possibility that rescuing may be due to a chaperone effect exerted by the interactors on the mt tRNAs structure altered by mutation.

The possibility that a small peptide could correct defects associated with many mt tRNA mutations suggests a novel therapy for mitochondrial diseases treatment. The involvement of the mt EF-Tu in cellular heat stress response has also been suggested.

Keywords: *mitochondrial diseases, tRNA mutations, suppressor genes*

[PS12-15] Identification of Steroidal Compounds with Anticancer Properties Using Yeast-based Bioassays

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Breast cancer is the most frequent cancer among women and one of the leading causes of mortality worldwide. Proliferation of breast cancer and other hormone-sensitive cancers like ovarian, prostate and endometrial is directly influenced by circulating levels of steroidal hormones. Those levels can be reduced by inhibition of enzymes involved in steroidogenesis, such as cytochrome P450s (CYP19, CYP17) and steroid oxidoreductases (17beta-HSD, AKR1Cs). Another treatment strategy is to target hormone receptors and prevent binding of hormones or modulate signaling; steroidal drugs such as fulvestrant bind estrogen receptors preventing receptor dimerization and translocation to the nucleus while accelerating receptor degradation. Still, prevalence of cancers resistant to current treatments makes development of new compounds with improved anticancer properties a priority. To test the ability of synthesized compounds or plant extracts to bind steroid hormone receptors, we optimized a non-transcriptional fluorescent cell assay in yeast. We then measured the relative affinities of these compounds for estrogen receptor alpha, estrogen receptor beta or androgen receptor ligand binding domains. Ligand binding domains of steroid receptors were expressed in-frame with yellow fluorescent protein in the yeast Sacharomyces cerevisiae. Addition of known steroid ligands to yeast expressing the appropriate cognate receptor results in increased fluorescence intensity, enabling estimation of receptor binding affinities in a dose-response and time-dependent manner. Relative binding affinities were evaluated by live cell fluorimetry and fluorescence microscopy and compounds were further characterized by in vitro cytotoxicity studies using human cancer cell lines. In vitro enzymatic assays and in silico molecular docking studies support our results and were also used to pre-select compounds for screening. In addition to identification of new ligands for steroid receptors, this assay could also be used to filter out compounds with potential for off-target interactions with steroid receptors during the early stages of compound screening. This combined approach is low-cost, reusable, and non-radioactive and has applications not only for drug screening, but also for detection of environmental chemicals with estrogenic/androgenic properties, and understanding the structural basis of steroid ligand recognition.

Keywords: *breast cancer, yeast assay, fluorescence*

[PS12-16] Using a drug-sensitive yeast strain as a model system to identify targets of antiparasitic and antineoplastic compounds

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Major advances in miniaturized phenotypic screening have resulted in the discovery of tens of thousands of new compounds with new but often uncharacterized mechanisms of action. Developing efficient ways to match these compounds to their targets remains a key challenge, especially for compounds with primary activity against eukaryotic pathogens with limited genetic toolboxes, such as the malaria parasite, Plasmodium falciparum or the etiological agent of Chagas disease, Trypanosoma cruzi.

Our approach utilizes a sensitized, attenuated strain of Saccharomyces cerevisiae, in vitro evolution and whole genome sequencing. By analyzing how yeast genetically adapts to treatment with small cytotoxic molecules we identify the compounds' targets and/or targeted pathways, predict resistance mechanisms, and define sites of compound/protein interactions. S. cerevisiae cells were exposed to over 70 compounds that are cytotoxic growth inhibitors (IC50< 80 uM) with known and unknown mechanisms of action and the evolved lines were subjected to whole genome sequencing to identify the mutations causative for the resistance phenotypes. For 9 compounds with known mechanisms of action, including cycloheximide, etoposide, the antimalarial KAE609, miconazole, fluconazole, posaconazole, etoposide, carmaphycin, and everolimus, the predicted target or pathway was identified in 8 cases. In addition, we obtained resistant strains against a subset of antiparasitic compounds. Whole genome sequencing identified putative targets and pathways, including targets involved in the cAMP and ergosterol biosynthesis pathway. General resistance mechanisms including mutations in key transcription factors were also identified. In addition, the location of resistance-conferring mutations on predicted protein structures provided insight into compound-protein interactions at amino acid resolution. Our extensive comparative-genomics approach expands on the limited number of tools available for analyzing compound-target interactions and can be applied to studies of other eukaryotic antimicrobials and chemotherapeutics.

Keywords: *yeast evolution, drug discovery, drug target identification*

[PS12-17] Analysis of interactions between mammalian amyloids in yeast Saccharomyces cerevisiae

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Amyloids are fiber-like ordered aggregates, generated via intermolecular cross-beta interactions. Amyloid fibrils and amyloid-related protein aggregates are associated with a variety of human diseases, including such widespread disorders as Alzheimer disease (AD), Parkinson's disease (PD), transmissible spongiform encephalopathies (TSEs) such Creutzfeldt-Jakob disease (CJD), type 2 diabetes (T2D), etc. Existing data demonstrate that some amyloids, produced by different proteins, can interact to each other. Co-localization of various aggregates has been demonstrated for such proteins as PrP (mammalian prion protein, associated with TSEs), tau and amyloid beta peptide (Abeta) (associated with AD). Several reports described simultaneous presence and colocalization of Abeta and PrP aggregates in CJD patients. Epidemiological studies point to increased incidence of AD

among the patients with T2D (associated with aggregation of the IAPP peptide). Previously, we have shown that yeast Saccharomyces cerevisiae is a reliable model system for studying interactions between different mammalian amyloids. By using yeast, we have confirmed direct interaction between PrP and Abeta aggregates, and identified the PrP sequences that are essential for the interaction. Here, we apply a yeast model to studying interactions between IAPP and Abeta, tagged CFP and YFP. Chimeric amyloidogenic proteins form detergent resistant aggregates in yeast cells. By using FRET, we demonstrate that Abeta and IAPP both co-localize and physically interact to each other. To our knowledge, this is the first evidence of direct interaction between IAPP and Abeta in vivo. We hypothesize such an interaction may play an important role in seeding Abeta aggregation in the T2D patients, resulting in AD development.

This work was supported by grants from RSF (14-50-00069) and RFBR (15-04-08159). Authors acknowledge the SPbSU Resource Centers «Biobank», «CHROMAS» and «Molecular and Cell Technologies» for technical support.

Keywords: *yeast, mammalian amyloids interactions, IAPP, amyloid beta peptide, FRET*

[PS12-18] Characterization in Saccharomyces cerevisiae of PTEN-L, an Alternative Translation Variant of the Human Tumour Suppressor PTEN

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Mammalian class I phosphatidylinositol 3-kinases (PI3Ks) are heterodimers consisting of a regulatory subunit and a catalytic subunit, which catalyse the conversion of the phosphoinositide PIP2 into PIP3 at the plasma membrane. The PIP3 acts as a second messenger favouring cell proliferation and inhibiting apoptosis and, therefore, its abnormal hyperactivation leads to tumorigenesis. The tumour suppressor PTEN is a lipid phosphatase that dephosphorylates PIP3 giving rise to PIP2, thus counteracting the activity of PI3K. We have developed a humanized yeast model by heterologous expression of PI3K and PTEN in Saccharomyces cerevisiae. The expression of active versions of the catalytic subunit of PI3K under the control of the GAL1 inducible promoter led to the yeast growth inhibition, while the co-expression of PTEN suppressed this phenotype (Rodríguez-Escudero, et al. 2005).

It has been recently reported that cells can produce a longer form of PTEN by alternative translation initiation, named PTEN-L, which can be secreted and internalized by recipient cells, exerting there its tumour suppressor function (Hopkins et al., 2013; Leslie and Brunton, 2013). We have found that PTEN-L expression in yeast led to several size variants of PTEN that also counteracted PI3K-dependent growth inhibition. GFP-PTEN-L localized sharply at the yeast plasma membrane (PM) and the nucleus. We show that this location is dependent on particular structural domains of PTEN-L and the presence of PIP2 at the PM.

By reducing PTEN-L expression in yeast, we have optimized our system aiming to the detection of gain-of-function PTEN-L mutations that display an enhanced rescue of PI3K-dependent growth inhibition.

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Keywords: *Humanized yeast, Tumour suppressor, PTEN, Plasma mebrane, Phosphoinositides*

[PS13] Medically relevant yeasts and host microbe interactions

[PS13-1] Using the Force in Yeast Biofilms: A Role for Cell Surface Amyloid-like Aggregation in Response to Shear Force

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Many biofilm-associated adhesins in bacteria and fungi can form amyloids. In yeasts, glycoproteins such as Saccharomyces cerevisiae Flo1 and Flo11 flocculins and Candida albicans Als adhesins can form amyloids in vitro, and in vivo they aggregate into cell-surface patches through interactions with amyloid-like properties: birefringence, binding of amyloid dyes, perturbation by anti-amyloid agents. The resulting cell surface clusters of adhesins can proliferate around the cell. The clustered adhesins mediate strengthened cell-substrate and cell-cell binding, both through increased binding avidity and through formation of amyloid-like interactions in trans between cells. These interactions form under flow, leading to increased cell binding and aggregation (“catch-bonding”), then to biofilm growth and persistence under flow. Anti-amyloid compounds inhibit both cellular aggregation and biofilm

To determine whether the amyloid-like properties of fungal surface nanodomains are sequence-specific, we have asked whether a disease-associated amyloid core sequence has properties equivalent to the native sequence in a fungal adhesin. We substituted the amyloid core sequence LVFFA from human Aβ protein for the native sequence IVIVA in the 1419-residue Candida albicans adhesin Als5p. The chimeric protein formed cell surface nanodomains and mediated increased cellular aggregation. The native-sequence and chimeric adhesins responded similarly to thioflavin T and to amyloid perturbants. Also, a peptide composed of the A amyloid sequence flanked by amino acids from the adhesin formed 2-dimensional sheets with sizes similar to the cell surface adhesin nanodomains. These results showed the similarity of amyloid-like interactions in the core sequences of native Als5p and Aβ, but also highlighted emergent properties of the native sequence. Unlike the native protein, the nanodomains formed by the chimeric protein were not force activated, and formed less robust aggregates under flow. Metadynamics modeling shows structural bases for the force-sensitivity in the native sequence adhesion and the lack of force-sensitivity in the chimeric adhesin. These results inform an initial model for structure and force-dependent activation of amyloid-like interactions in yeast adhesins.

Supported by PHS/NIGMS R01 098616

Keywords: *Cell wall, biofilms and mats, glycoproteins, flocculation and aggregation, functional amyloids*

[PS13-2] Comparative Genomic and Transcriptomic Analyses Unveil Novel Features of Azole Resistance and Adaptation to the Human Host in Candida glabrata

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The emergence of azole resistance among Candida glabrata strains is frequent and contributes to increase the incidence of infections caused by this species. In this work we aimed at elucidating the molecular mechanisms underlying resistance to fluconazole and voriconazole in a resistant clinical isolate (FFUL887). Whole-genome sequencing of FFUL887 and subsequent comparison with the genome of the susceptible reference strain CBS138 revealed the existence of prominent differences in several genes documented to promote azole resistance in C. glabrata. Among these was the transcriptional regulator CgPdr1. The CgPdr1 allele encoded by the FFUL887 strain included a K274Q modification not documented in other azole-resistant strains. The significant increase in susceptibility to azoles of the FFUL887 strain upon deletion of the CgPDR1K274Q allele, along with results from transcriptomic profiling rendering evident the upregulation of 80 documented targets of CgPdr1 in the FFUL887 strain, support the idea that K274Q is a novel CgPdr1 gain-of-function mutation. Analysis of the non-coding genome of the FFUL887 and of CBS138 support the idea that in the FFUL887 strain alterations of the CgPdr1-controlled regulatory network may have changed its architecture to improve the expression of azole-resistance genes. Comparison of the genome of the FFUL887 and CBS138 also showed prominent differences in the sequence of adhesin-encoding genes, while comparison of the transcriptome of the two strains showed a significant remodelling of the expression of genes involved in metabolism of carbohydrates, nitrogen and sulphur in the FFUL887 strain; these responses probably reflecting adaptive responses evolved by the clinical strain during colonization of the host.

Keywords: *antifungal resistance, comparative genomics and comparative transcriptomics, CgPdr1 transcription factor, Candida glabrata, fungal infections*

[PS13-3] Fruits are Vehicles of Pathogenic Yeasts

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We would like to investigate the diversity of pathogenic yeasts on fruit surfaces. We isolated and characterized yeast isolates recovered from fruit surfaces. A total of 184 isolates, comprised of 55 species, from 22 different types of fruits in markets in Taiwan were analyzed. Among them, 86 isolates, of 29 species, are reported to cause diseases in humans. They included Aureobasidium melanogenum, Aureobasidium pullulans, Candida famata, Candida fermentati, Candida guilliermondii, Candida intermedia, Candida krusei, Candida orthopsilosis, Candida parapsilosis, Candida pelliculosa, Candida tropicalis, Trichosporon asahii, and 17 other species. In addition to C. krusei, intrinsically resistant to fluconazole, all Rhodotorula and Rhodosporidium species were resistant to fluconazole. The two C. tropicalis isolates are, of respectively, of diploid sequence type (DST)149 and DST225, genotypes also detected in isolates from humans. Furthermore, the DST225 isolate was less susceptible to azole drugs (fluconazole MIC 4 mg/l and triadimenol MIC 8 mg/l). The susceptibilities to azole drugs for clinical and agricultural usage were associated to each other. It is important to be aware of the existence of pathogenic yeasts, especially drug resistant ones, on the fruit surfaces, a potential route for pathogenic yeasts to be transmitted to humans.

Keywords: *yeast diversity, pathogenic yeast, environment, drug susceptibility*

[PS13-4] Genetic Adaptive Mechanisms Mediating Response and Tolerance to Acetic Acid Stress in the Human Pathogen Candida glabrata: Role of the CgHaa1-dependent Signaling Pathway

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The increased resilience of Candida glabrata to azoles and the continuous emergence of strains resistant to other antifungals demands the development of new therapeutic approaches focused on non-conventional biological targets. Genes contributing to increase C. glabrata competitiveness in the different infection sites are an interesting and unexplored cohort of therapeutic targets. To thrive in the vaginal tract and avoid exclusion C. glabrata cells have evolved dedicated responses rendering them capable of tolerating multiple environmental challenges, including the presence of acetic and lactic acids produced by the commensal microbiota. In this work it is shown that the CgHaa1 transcription factor (ORF CAGL0L09339g) controls an acetic acid-responsive system essential for survival of C. glabrata in presence of acetic acid at a low pH. mRNA profiling showed that the genes up-regulated by CgHaa1 under acetic acid stress are involved in multiple physiological functions including membrane transport, metabolism of

carbohydrates and amino acids, regulation of the activity of the plasma membrane H+-ATPase and adhesion. Consistently, under acetic acid stress CgHaa1 increased the activity and the expression of the CgPma1 proton pump and enhanced colonization of vaginal epithelial cells by C. glabrata. The protective effect of CgHaa1 against acetic acid was also linked to the reduction of the accumulation of the acid inside C. glabrata cells, this being partly dependent on the up-regulation of the multidrug transporter CgTpo3. Comparison of the CgHaa1-dependent regulatory network active in C. glabrata with the corresponding Saccharomyces cerevisiae orthologue network revealed prominent differences, consistent with the idea that the two pathways have evolved divergently with the CgHaa1 pathway suffering a “functional expansion”. The role of the CgHaa1-pathway in the extreme acetic acid-tolerance exhibited by vaginal C. glabrata isolates will also be discussed.

Keywords: *stress response and signalling in C. glabrata, Transcriptional regulation, Evolution of regulatory networks, Acetic acid stress tolerance*

[PS13-5] Polygenic Analysis of High Acetic Acid Accumulation, a Novel Putative Probiotic Property of Saccharomyces cerevisiae var. boulardii

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Probiotics are microorganisms that confer beneficial properties on their mammalian host when ingested. Saccharomyces boulardii is the best established yeast probiotic1. Since it was isolated from lychee fruit by Henri Boulard in 1920, it has been used as a preventive and therapeutic agent against diarrhea. Numerous positive health effects have been attributed to its use. These include increasing the availability of nutrients in fermented food, degradation of pathogenic toxins and support to the immune system. It also increases short-chain fatty acid levels in the gut, with possible antibacterial action2. Upon investigation of a collection of S. boulardii strains, we noticed for some strains a large zone of bacterial growth inhibition in an agar-well diffusion assay with an E. coli indicator strain exposed to the cell-free supernatant of an S. boulardii culture. Investigation of these supernatants showed that they contained a high concentration of acetic acid. We performed quantitative trait locus (QTL) mapping by pooled-segregant whole-genome sequence analysis of S. boulardii acetic acid accumulation capacity. For that purpose, a diploid S. boulardii was made homozygous for mating type, crossed with a compatible S. cerevisiae diploid strain, and after two rounds of sporulation, a haploid segregant with a comparable acetic acid accumulation as the parental S. boulardii strain was isolated. It was then crossed with a haploid S288c strain. The hybrid was sporulated and 549 segregants were evaluated for acetic acid accumulation, after which 32 superior segregants were pooled and sequenced. Mapping of the SNP variant frequency revealed two major and some minor QTLs. They were analyzed by reciprocal hemizygosity analysis and for each major QTL a causative gene for acetate accumulation was identified. Although reciprocal allele exchange between S. boulardii and S. cerevisiae confirmed the causative character, additional important causative genes in minor QTLs appear to be present. This is currently being investigated in more detail. Overall, this methodology can be applied to investigate other traits linked to the probiotic action of S. boulardii.

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Keywords: *Polygenic analysis, Probiotic, Saccharomyces cerevisiae var. boulardii, High acetic acid accumulation*

[PS13-6] Structural and Functional Analysis of CFEM Proteins for Hemoglobin Iron Acquisition

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Iron acquisition from host tissues is a major virulence factor for a pathogenic microorganism. It is a particular challenge to the pathogenic microorganism due to the high-affinity iron-chelation mechanism developed by the host that limit its bioavailability and providing the host nutritional immunity. Many microbial pathogens have therefore evolved mechanisms for extracting iron from hemoglobin, the largest iron reservoir in the host. Candida albicans, a human commensal microorganism that can cause life-threatening systemic infections in immunocompromised individuals has evolved a pathway for heme-iron scavenging from hemoglobin. This pathway includes a relay network of secreted and GPI-anchored extracellular proteins containing a CFEM domain, which is defined by a sequence consisting of 8 cysteine residues with conserved spacing. These proteins can extract heme from hemoglobin outside the cell and transfer the heme across the cell envelope from one CFEM protein to the next until delivered to the endocytic pathway. The crystal structure of the secreted CFEM protein Csa2 reveals that the CFEM domain adopts a novel helical-basket fold consisting of six α-helices. The structure is stabilized by four disulfide bond between eight conserved cysteine residues. Site-directed mutants of these cysteines suggest they play no role in heme binding or transfer. The planar heme molecule is bound between a flat hydrophobic platform located on top of the helical basket and a flexible N-terminal loop. Uniquely, an aspartic acid residue serves as the axial heme-iron ligand. Mutational analysis of the Csa2 protein surface based on in-silico modeling identified mutants that are able to bind heme and extract it from hemoglobin, but that are defective in heme transfer. These mutants may identify a site of interaction between CFEM proteins that mediates heme transfer. Our results, which reveal the molecular details of the mechanism of this widely conserved fungal pathway used by the pathogen to overcome host nutritional immunity, may enable the development of novel antifungal therapies.

Keywords: *"Iron acquisition", "Candida albicans", "Nutritional immunity", "CFEM", "Heme-iron scavenging", "CSA2", "Helical-basket Model"*

[PS13-7] Cell Membrane as an Antifungal Target of Antimicrobial Peptides of the Halictines Family

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The fungal plasma membrane and cell wall serve as the armour of yeast cell – protect the cell against toxic agents including antifungals, determine the cell shape and provide mechanical strength to oppose turgor pressure. At the same time they are also the fungus weaknesses since their major components are absent from mammals and can be specifically targeted with compounds that do not affect human metabolism as seen with antifungal drugs that are targeted against ergosterol (polyenes) and various steps of its biosynthesis (imidazoles and triazoles, allylamines and thiocarbamates, morpholines) or the synthesis of β-1,3-glucan (echinocandines). Widespread and prolonged use of antifungals in recent years has led to the rapid emergence of multidrug resistant strains of Candida. Therefore novel treatments for fungal infections are urgently needed. Naturally occurring antimicrobial peptides are promising candidates for treatment of fungal infections.

We compared the potency of four derivatives of the antimicrobial peptide halictine-2 against six Candida species. Their efficacy was linked to their structure and the specific Candida species. Halictines rapidly permeabilized cell membranes and caused the leakage of cytosolic components resulting in cell death. Their fast mechanism of action can overcome multidrug resistance, since their effect on C. glabrata cells did not depend on the activity of Cdr1 pump, but was influenced by lipid composition of the plasma membrane. Diminished ergosterol content due to terbinafine or fluconazole pretreatment caused resistance to the peptides. On the other hand, an enhancement of the peptides' action was observed when cells were pretreated with myriocin, which blocks the synthesis of sphingolipids. Their killing potential was also increased when the peptides were used together with the surface-active antimicrobial agent octenidine dihydrochloride or in combination with amphotericin B.

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Keywords: *antimicrobial peptides, Candida, diS-C3(3) assay*

[PS13-8] Bacterial Signalling Nucleotides Inhibit Yeast Cell Growth by Impacting Mitochondrial and other Specifically Eukaryotic Functions

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We have engineered Saccharomyces cerevisiae to inducibly synthesise the bacterial signalling nucleotides cdiGMP, cdiAMP and ppGpp in order to characterize the range of effects these nucleotides exert on eukaryotic cell function. Synthetic genetic array (SGA) and transcriptome analyses indicate that, while these compounds elicit some common reactions in yeast, there are also complex and distinctive responses to each of the three nucleotides. All three are capable of inhibiting eukaryotic cell growth, with the guanine nucleotides exhibiting stronger effects than cdiAMP. Mutations compromising mitochondrial function and chromatin remodelling show negative epistatic interactions with all three nucleotides. In contrast, certain defects in chromatin modification and ribosomal protein function show positive epistasis, alleviating growth inhibition by at least two of the three nucleotides. Uniquely, cdiGMP is lethal both to cells growing by respiration on acetate and to obligately fermentative petite mutants. cdiGMP is also synthetically lethal with the ribonucleotide reductase (RNR) inhibitor, hydroxyurea. Heterologous expression of the human ppGpp hydrolase, Mesh1p, prevented the accumulation of ppGpp in the engineered yeast and restored cell growth. Extensive in vivo interactions between bacterial signalling molecules and eukaryotic gene function occur, resulting in outcomes ranging from growth inhibition to death. cdiGMP functions through a mechanism that must be compensated by unhindered RNR activity or by functionally competent mitochondria. Mesh1p may be required for abrogating the damaging effects of ppGpp in human cells subjected to bacterial infection.

Keywords: *Signalling nucleotides, Pathogenesis, Purine nucleotides, Cell-cell interactions*

[PS13-9] Effects of New Synthesize Anticandidal Compounds on Ergesterol Biosynthesis

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In the past two decades, a dramatic rise has been observed in the incidence of fungal infections due to increasing number of immunocompromised hosts. Among them Candidiasis can range from non-life threatening mucocutaneous infections to incursive progressions. Since antibiotic resistance is a dramatically growing public concern, the risk of untreatable infections is quite high. To overcome this challenge, there has been extensive research on the synthesis of novel antibiotics and modifying the structure of existing antibacterial agents is another approach as well. With these strategies, several drugs discovered and improved. As a most practical solution, the second strategy attracts more attention. Here, with this work we synthesized new benzimidazole derivatives. Benzimidazole compounds have always been important pharmacophores in studies on new antimicrobial agent development. Among 29 new synthesize compound, 7 of them showed better antifungal activity than reference drug. Two of these 7 compounds were not cytotoxic. Effect of these two compounds on ergesterol biosynthesisi evaluated by LC-MS-MS. We determined ergesterol amount of C. albicans which exposed these two compound. Results showed that the decline in ergosterol levels after treatment with compounds was noticeable compared to the reference agents.

Keywords: *Candida albicans, antifungal, ergesterol*

[PS14] Yeast and industrial biotechnologies

[PS14-1] Engineering Cofactor and Precursors Supply in Saccharomyces cerevisiae for Enhanced triterpene Biosynthesis

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Engineering robust cell factories to effectively produce a desired product at high titer, rate, and yield while dealing with industrially relevant stresses is usually the most challenging step in the development of industrial production of chemicals using microbial fermentation processes. Synthetic biology and metabolic engineering enable generation of novel cell factories that efficiently convert renewable feedstocks into fuels and high-value chemicals, thus creating the basis for biosustainable economy independent on fossil resources. Here we engineered and constructed the yeast platform cell factory for the robust production of high-value pharmaceutical compounds, triterpenes produced by some plants in trace amounts. We applied a pull-push-block strain engineering strategy that included overexpression of the triterpene biosynthesis pathway, modulation of redox balance, and increase of the precursors supply. The engineered strain achieved higher triterpene production when compared to a strain expressing only triterpene biosynthesis pathway. The yeast strains engineered in this work can serve as the basis for creating an alternative way for producing triterpenes in place of extractions from plant sources.

Keywords: *Triterpene, Saccharomyces cerevisiae, Metabolic engineering*

[PS14-2] Ethyl acetate production by the elusive alcohol acetyltransferase from yeast

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Ethyl acetate is an industrially relevant ester that is currently produced exclusively through unsustainable processes. Many yeasts are able to produce ethyl acetate, but the main responsible enzyme has remained elusive, hampering the engineering of novel production strains. Here we describe the discovery of a new enzyme (Eat1) from the yeast Wickerhamomyces anomalus that resulted in high ethyl acetate production when expressed in Saccharomyces cerevisiae and Escherichia coli. Purified Eat1 showed alcohol acetyltransferase activity with ethanol and acetyl-CoA. Homologs of eat1 are responsible for most ethyl acetate synthesis in known ethyl acetate-producing yeasts, including S. cerevisiae, and are only distantly related to known alcohol acetyltransferases. Eat1 is therefore proposed to compose a novel alcohol acetyltransferase family within the α/β hydrolase superfamily. The discovery of this novel enzyme family is a crucial step towards

the development of biobased ethyl acetate production and will also help in selecting improved S. cerevisiae brewing strains.

Keywords: *Ethyl acetate, yeast, alcohol acetyltransferase, Saccharomyces cerevisiae, α/β hydrolase, Escherichia coli*

[PS14-3] Single-Cell Sensors of Physiology as Burden Sensors for Synthetic Biological Circuits

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Saccharomyces cerevisiae is one of the best understood and most engineered microbial cell factories being used in the production of high-value compounds, drugs and biofuels. Introducing heterologous genetic circuits and metabolic pathways often cause stress on the cell, diminishing product yields and reducing cell growth. Therefore, past efforts focused on fine-tuning expression levels to lower metabolic burdens and increase yields.

Our focus lies on the production of plant-derived high value compounds in yeast. Saponins are glycosylated triterpenes with numerous potential applications across the pharmaceutical, home and personal care, agriculture, food and beverage sectors. Current challenges in include the toxicity of saponins and their precursors as well as the balancing the ratios of cytochrome P450 reductases and cytochrome P450-dependent monooxygenases.

We aim to monitor the stress response on the single-cell level. We used A Long-term Culturing And TRApping System (ALCATRAS) developed in our lab to trap single yeast cells in a microfluidic device and study them over long periods of time (up to 24 hours) in a controlled environment. We tracked nuclear localisation of GFP-tagged stress response factors (Msn2, Hog1, Yap1, Mpk1) in response to the induction of various genetic circuits by fluorescence microscopy. We were able to identify burdens introduced by heterologous metabolic pathways and plan to counter them with fine-tuned gene expression or the activation of endogenous stress response pathways. Our results will significantly contribute to the understanding of yeast stress response and enable the rational design of stress-tolerant strains for the microbial production of high-value compounds.

Keywords: *Single-Cell Sensors, Microfluidics, Stress Response, Metabolic Engineering, Synthetic Biological Circuits, Plant-derived Natural Products*

[PS14-4] Nutritional Requirements Of Wine Yeast Strains: Role Of The TORC1 Pathway On Winemaking Conditions

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Wine alcoholic fermentation is mainly carried out by enological strains of Saccharomyces cerevisiae, hence the importance of the environmental and genetic factors that determine their growth and aging, not only for their relevance on the fermentative process but also by their impact on wine organoleptic properties. When a nutrient is scarce, dietary restriction happens, which consists in the reduction of nutrients supply without inducing malnutrition. Dietary restriction controls longevity through nutrient signaling pathways, as the TORC1 pathway, which promotes growth and protein synthesis when nitrogen sources are abundant. As sugars are plenty in grape juice, generally is nitrogen the limiting nutrient. Thus, the study of the physiological processes by which a selective nitrogen source is used is of great importance. Hence, our interest in TORC1 targets, such as ribosomal Rps6 protein and the nitrogen catabolic repression (NCR) system, that represses the use of poor nitrogen sources when optimal sources are present. Its main effectors are GATA transcriptional factors (Gln3 and Gat1), controlled mainly by TORC1.

The behaviour of NCR transcription factors Gat1 and Gln3 is being studied in wine strains. Our results indicate genetic differences between wine and laboratory yeast strains, mainly in their response to nitrogen shortage. Indeed, using phosphorylation of Rps6 as a tool to study TORC1 activity, our data indicate that wine and laboratory yeast strains behave differently against several TOR inhibitors, such as rapamycin or methionine sulfoximine (MSX). Moreover, TORC1 pathway seems to be only active in the first hours of winemaking, indicating that signalling of nitrogen shortage occurs earlier than expected. Under these conditions, other nutrient signalling pathways (Ras/AMPc/PKA or Snf1) could be active to promote growth, and that aspect is being also addressed. In general, TORC1 pathway can integrate the nitrogen signal and coordinate other pathways to respond to the specific environmental winemaking conditions.

Keywords: *Wine yeast, TORC1 pathway, Nitrogen catabolic repression (NCR)*

[PS14-5] Construction of the flavinogenic yeast Candida famata overproducing riboflavin, flavin nucleotides and bacterial fravin antibiotics aminoriboflavin and roseoflavin

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Flavinogenic yeasts overproduce riboflavin under iron limitation. Candida famata belongs to this group and is one of the most flavinogenic yeasts known. Earlier, this species was used for construction of the industrial riboflavin producers; however, they appeared to be unstable so corresponding process was shut down several years ago. Recently, we have constructed stable riboflavin overproducer of C. famata using combined approaches of metabolic engineering and classical selection. This was achieved due to overexpression of the gene SEF1 coding for transcription activator of the structural genes of riboflavin pathway, gene IMH3 coding for IMP dehydrogenase and selection for resistance to several antimetabolites, mainly structural analogs of purines and riboflavin. Riboflavin

overproducer was used for construction of the strains overproducing flavin nucleotides FMN and FAD due to overexpression of the genes FMN1 and FAD1 coding for riboflavin kinase and FAD synthetase, respectively. There is known the antibiotic of flavin nature, roseoflavin, produced by the soil actinomycete Streptomyces davawensis. Roseoflavin is effective against pathogenic Gram-positive bacteria including methicillin-resistant Staphylococcus aureus. It was found recently that roseoflavin is synthesized from FMN through aminoriboflavin. We decided to construct yeast C. famata producing aminoriboflavin and roseoflavin. For this, C. famata gene FMN1 and S. davawensis genes rosB and rosA were overexpressed under control of strong constitutive TEF1 or inducible MAL2 promoters. Strains of C. famata with overexpression of the own FMN1 gene and the heterologous rosB gene accumulated aminoriboflavin (biosynthetic precursor of roseoflavin). Strains which co-overexpress FMN1, rosB and rosA genes have also been constructed. Apparently this is the first evidence for successful construction of the yeast strains producing bacterial antibiotici. Perspectives of the construction of the competitive producers of riboflavin, flavin nucleotides and flavin antibiotics are discussed.

Keywords: *flavinogenic yeast, Candida famata, riboflavin, flavin coenzymes, FMN, FAD, flavin antibiotics, roseoflavin, aminoriboflavin, metabolic engineering*

[PS14-6] Draft Genome Sequence of the Multi-Stress Tolerant Yeast Pichia farinosa

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The strain Pichia farinosa KCTC27753, isolated from Nuruk, is a multi-stress tolerant yeast that could grow at 46℃ and pH 3.0. This strain shows possibility for producing bioethanol because it can withstand the fermentation inhibitors including furfural and phenolic compounds released from biomass. Whole genome sequence of P. farinosa KCTC27753 was analyzed using the PacBio RSII. The draft genome sequence of this strain was 21,255,474 bp length with G+C content of 41.09%, consisting of 17 contigs. Total, 10,910 plausible gene-coding regions were identified and the BioProject has been deposited at DDBJ/ENA/GenBank as PRJNA369593.

Keywords: *genome sequence, multi-stress tolerance, Pichia farinosa, Nuruk*

[PS14-7] Lipid Production From Waste Materials by the Genetically Modified Yeast Yarrowia Lipolytica

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Nowadays, biodiesel is still the primary renewable alternative to diesel. Fossil fuels are likely to soon be exhausted and plant energy resources will compete with food production for farmland. Microbial lipids might become one of potential oil feedstock for biodiesel

production from low-cost carbon sources like crude glycerol or lignocellulose materials. Glycerol is produced by several industries for example fat saponification or biodiesel production. Approximately 1 kg of crude glycerol is generated per every 10 kg of biodiesel produced. Crucial to the economic worthwhile of these process is production high lipid titres by properly prepared strains. Yarrowia lipolytica is well-known oleaginous microorganism, the most studied and engineered in recent years. This yeast has been classified as a GRAS organism and proven suitable for many industrial process. Lipid synthesis in cells depends on activity of various enzymes. In this study, we aimed to examined the impact of overexpression of few different genes involved in fatty-acid synthesis and metabolism of various carbon feedstock. To enhance production of single cell oils from glycerol we overexpressed the GUT1 (YALI0F00484g) gene coding glycerol kinase and to direct carbon flow into lipid production we overexpressed the SCT1 (YALI0C00209g) gene encoding G3P-acyltransferase. We additionally overexpressed DGA1 (YALI0E32769g) gene encoding DAG-acyltransferase involved in the last step of triglycerides synthesis. In addition we evaluated the impact of medium composition and culture condition on lipid content. Finally, we obtain strains that accumulated up to 50% of their DW as lipid. Improved lipid content and lipid yield were successfully achieved. This work demonstrates that effective metabolic engineering may create biological platform for efficient lipid production by yeast and biosynthesis pathway are subject to multi-level influence. This is a relevant step towards to development of cost-effective process for biodiesel green production from renewable resources.

Keywords: *microbial oils, Yarrowia lipolytica, industrial wastes, metabolic engineering*

[PS14-8] Impact of Reuse of Saccharomyces cerevisiae in the Fermentation Performance on an Industrial Brewery

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There is in vitro evidence that industrial brewery yeast handling is connected with a decrease on the physiological state, leads to poor fermentation performance, low-quality beer, and contamination events. Despite these, the impact on fermentation performance during the reuse of yeast in the brewing industry remains unclear. Our approach consist in links the fermentable carbohydrates rate consumption, ethanol and volatile compounds (acetaldehyde, ethyl acetate, isoamyl alcohol and diacetyl) production, on top fermented beer, with the physiological state of reused brewing strain Saccharomyces cerevisiae in terms of vitality, viability, trehalose and glycogen concentration, flocculation potential and cell wall compounds. We have analyzed subsequent fermentations on brewery from new slurry yeast until last recycles of beer production, observing a decrease in viability, vitality and concentration of trehalose, while there is an increase in flocculation potential. Carbohydrates from wort consumption pattern varied between new and repitching yeast. We observe diverse morphological patterns from the original cell yeast: abnormal small cell yeast positives to methylene blue staining test and cluster-forming yeast. We consider with

the obtained data that during yeast repitching, stress factors produced on industrial beer production affect the ability of the cell yeast to adapt brewery condition, based on the numbers of repitching; producing a positive feedback between the decrease of the physiological condition and the variation of fermentation performance.

Keywords: *Repitching yeast, Physiological state, Beer, Fermentation performance, Alcohol & volatile compounds*

[PS14-9] Association between Alcoholic Fermentation and S-adenosylmethionine in Beer Brewing

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In lager beer brewing, the fermentation performance of the bottom-fermenting brewer's yeast Saccharomyces pastorianus impacts beer quality. In recent years, strains suited to high-gravity brewing have been favored because they reduce costs and energy as well as permit the production of new types of beer such as those with high alcohol content. Conditions of high gravity, high osmotic pressure, and high alcohol concentration are stressful for brewer's yeast, resulting in a fermentation delay during high-gravity brewing. Thus, the breeding or selection of yeast with increased fermentation rates in high-gravity wort is highly desirable. We sought to identify factors that govern the fermentation ability of such strains. We focused on a sake yeast strain of Saccharomyces cerevisiae that is known to yield higher levels of alcohol (≧20% (v/v)) during sake fermentation. Recent studies showed that a defective transition to the G0 phase is one reason why sake yeast produce high concentrations of alcohol (Watanabe et al., J. Biosci. Bioeng. 2011; Watanabe et al., Appl. Environ. Microbiol. 2012; Watanabe et al., Appl. Environ. Microbiol. 2016). We previously constructed recombinant strains of bottom-fermenting brewer's yeast with improved fermentation rates of by applying knowledge gained from sake yeast (Oomuro et al., J. Biosci. Bioeng. 2016). However, beers brewed using recombinant strains are disfavored in the Japanese market; beers brewed with traditionally bred (non-genetically modified) yeast would be more appealing. Here, we describe work focused on fermentation metabolites of yeast. Recent studies of sake yeast revealed the accumulation of high levels of S-adenosylmethionine (SAM) (Kanai et al., J. Biosci. Bioeng. 2017) in sake yeast cells. Other work has demonstrated that SAM is involved in regulating glycolysis (Li et al., Mol. Cell 2015). We therefore focused on the relationship between SAM levels and alcohol fermentation by bottom-fermenting brewer's yeast. High-gravity fermentation of SAM-supplemented wort, or the use of yeast able to accumulate high levels of SAM, provided increased fermentation rates compared to those of controls. These results suggested that intracellular SAM accumulation is associated with enhanced fermentation by bottom-fermenting brewer's yeast. Thus, breeding for increased intracellular SAM accumulation in brewer's yeast may facilitate the engineering of strains better suited to high-gravity brewing.

Keywords: *Alcoholic fermentation, S-adenosylmethionine, Saccharomyces pastorianus, ADO1*

[PS14-10] Characterization of Transcriptional Regulatory Proteins in Pichia pastoris

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The methylotrophic yeast Pichia pastoris (syn Komagataella spp) plays a major role in the synthesis of high amounts of recombinant proteins for biopharmaceutical or industrial purposes. However, many complex secretory proteins still cannot be produced at the desired high levels during industrial production processes. Concerted engineering of the cellular response by modulation of transcriptional regulatory proteins has proven to be a promising approach to increase productivity in P. pastoris (Delic, et al., Microbial Cell, 2014; Gasser, et al., Biotechnol Bioeng, 2006; Guerfal, et al., Microb Cell Fact, 2010; Ruth, et al., Microb Cell Fact, 2014).

Gene expression is regulated at various levels, including epigenetic and transcriptional control of gene expression in response to external signals. The endowment of a given organism with transcriptional regulatory proteins, which regulate distinct gene sets in response to different signals, has a strong influence on its regulatory behavior and phenotype. Up to now, a large number of transcriptional regulators present in P. pastoris are of uncharacterized function, meaning that their application to targeted cell engineering is limited.

To shed light onto regulatory targets of yet uncharacterized transcriptional regulators in P. pastoris CBS7435, transcription factors knock-outs were generated using CRISPR/Cas9-based homology-directed genome editing. The strains obtained were cultivated on media representing different growth and stress conditions. In addition to growth ability in the presence or absence of stressors and different nutrient sources, morphology of the strains on their macroscopic and microscopic level was embraced.

Keywords: *transcription factors, Pichia pastoris, CRISPR/Cas9 based genome engineering*

[PS14-11] Chemogenomic Analyses Unveils New Players Governing Saccharomyces cerevisiae Tolerance To The Add-Value Itaconic And Levulinic Carboxylic Acids

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The carboxylic acids levulinic acid (LA) and itaconic acid (IA) have the potential to be used as building blocks in chemical synthesis replacing oil-derived catalysts. Microbe-based production of LA and IA is envisaged in an integrated biorefinery context to diversify the set of compounds that can be produced from biomass. Microbial production of LA has not

been implemented but for IA several strategies have now been implemented, mainly exploring the biochemical pathways active in strains that naturally produce this acid. The production of IA, LEV or of carboxylic acids in general is limited, among other factors, by the deleterious effects that these molecules produce over the producing cells specially at the later stages of the fermentation where the acids accumulate at high concentrations in the acidic broth. LA is also frequently found in lignocellulosic hydrolysates being an important fermentation inhibitor. To gain knowledge into the toxicity mechanisms imposed by ITA and LEV acids at a low pH in S. cerevisiae a large-scale phenotypic screening was performed using the Euroscarf haploid deletion mutant collection. According with the results obtained, 430 genes were identified as determinants of resistance to IA while for LA 296 were identified, with 42 genes contributing to S. cerevisiae resistance to both IA and LA. Furthermore, 18 genes whose expression was found to confer susceptibility to IA were also identified. Among the genes whose expression was found to contribute for maximal S. cerevisiae tolerance to IA or LA were several plasma membrane transporters which were hypothesized to serve as putative exporters for the anions of these carboxylic acids. Further analysis indeed confirmed the involvement of one of these transporters in mediating the export of IA. Other identified ITA-resistance genes were found to be involved in carbohydrate metabolism, in plasma membrane lipid composition, in vesicular transport and in stress response. In the case of LA it was visible the involvement of genes involved in amino acid and carbohydrate metabolism and vesicular transport. The results obtained led to the identification of a set of genes/functions that could be modulated to improve tolerance to ITA and LEV aiming to obtain more robust strains that could be used as cell factories for the production of these acids.

Keywords: *itaconic acid, levulinic acid, biorefinery, disruptome*

[PS14-12] Characterization of a Combinatorial Xylose Utilization Library in Saccharomyces cerevisiae in Ligno-cellulosic Biomass Hydrolysates

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Improving Xylose utilization is essential for the sustainable and economically feasible production of biofuels and bioproducts from lignocellulosic sources.

In recent years, much progress has been made in identifying heterologous and native genes beneficial for Xylose assimilation in Saccharomyces cerevisiae. However, the relevant variables leading to optimal gene expression and thus optimal results in different growth conditions remain poorly understood.

Here, we employ a combinatorial assembly approach of a genomically integrated Xylose assimilation pathway (Xylose transporter HXT7(F79s), Xylose Isomerase XI, Xylulokinase XKS1 and Transaldolase TAL1 involved in the Pentose-Phosphate pathway) by using a set of seven promoters of varying expression levels and expression patterns as well as three different terminators to regulate each gene. We characterized the resulting strains under different industrially relevant conditions and analyzed for optimal strain performance.

Keywords: *Xylose utilization, Ionic Liquids, Hydolysates, Biomass, Combinatorial Assembly*

[PS14-13] Higher Antioxidant Defences Result In A Better Industrial Performance In Non-Saccharomyces Wine Yeasts

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In recent years there has been a growing interest in the usage of non-Saccharomyces yeasts as co-inoculums in the wine industry. There have been many reports in which different species have been used in mixed fermentations alongside Saccharomyces cerevisiae. Their ability to produce secondary metabolites, reduce alcohol content or their specific enzymatic activities affect the wine profile, resulting in wines with enhanced and more complex flavours and aromas, which makes them important biotechnological tools. Most of these non-Saccharomyces yeasts occur naturally the environment, and although there are already commercially available strains, most inoculums have not been produced at an industrial scale.

We aim to characterize the behaviour and redox state of a set of non-Saccharomyces yeasts during yeast biomass propagation and dehydration, the two main industrial processes in active dry yeast production. We also intent on understanding the effects of the oxidative stress associated with these processes in their industrial performance. Another objective is to isolate new potentially interesting non-Saccharomyces yeast strains from other kind of drinks, Ecuadorian chichas, and evaluate their stress tolerance.

In this study we used a set of biochemical and physiological parameters that allowed us to determine the redox state of the studied yeasts, amongst them we analysed trehalose and gluthatione levels, gluthatione reductase and catalase activity, and lipid peroxidation. In order to analyse the industrial performance of our set of yeasts we measured the fermentative capacity and their viability after dehydration. Our results show high variability between the studied yeasts, those with increased antioxidant defences present a better tolerance and a reduction in fermentative capacity loss after dehydration, due to reduced oxidative damage. Other stress responses were studied in the newly isolated chichas yeasts to better understand the adaptation to their particular environment.

Keywords: *wine yeast, non-Saccharomyces, stress, antioxidant, dry yeast*

[PS14-14] Enhancing Heterologous Protein Secretion in Cellulolytic Saccharomyces cerevisiae Strains

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A major obstacle to using the yeast Saccharomyces cerevisiae in single-step hydrolysis and fermentation of cellulosic material for second generation bio-ethanol production is its

inferior yields of secreted heterologous cellulases. We have attempted to enhance heterologous protein secretion through rational design strategies involving several proteins integral to the secretion pathway. 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. We have demonstrated an increase in secretory titers for the Talaromyces emersonii Cel7A (a cellobiohydrolase) and Saccharomycopsis fibuligera Cel3A (a β-glucosidase) expressed in Saccharomyces cerevisiae through single and co-overexpression of some of the “ER-to-Golgi” and “exocytic” SNARE components. We conclude that SNARE proteins fulfil an essential role within a larger cascade of secretory machinery components that could contribute significantly to future improvements to S. cerevisiae as protein production host. As heterologous protein secretion was previously shown to induce cellular stress in yeast, we also investigated the role of stress response genes in successful heterologous protein production. We have demonstrated that overproduction of certain stress related enzymes could enhance heterologous cellulase production by over 2-fold. Finally we will report how a strain breeding approach was used to generate yeast strains for improved cellulase secretion. These results demonstrate the unexploited potential of S. cerevisiae as heterologous protein production host.

Keywords: *cellulosic ethanol, consolidated bioprocessing, enzymatic hydrolysis, yeast secretion pathway, cellobiohydrolase expression, rational strain design*

[PS14-15] Identifying Gene Targets for Improving L-Phenylacetylcarbinol Production in Saccharomyces Cerevisiae by In-Silico Aided Metabolic Engineering and In-Vivo Validation

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The use of microbes in industrial processes requires modification of natural metabolism in order to enhance efficiency and economic yield. As the cellular metabolism is a complex system, finding the best target genes whose manipulations can improve the biosynthesis of a desired product is not straightforward. Hence in metabolic engineering, whole metabolic network should be analyzed for identifying genes that affect the product formation. L-Phenylacetylcarbinol (L-PAC) is a precursor of several decongestant and antiasthmatic medicines such as ephedrine and pseudoephedrine. In yeast cells, pyruvate decarboxylase (PDC) is able to catalyze the condensation of exogenously added benzaldehyde and endogenously produced pyruvate into L-PAC. Alcohol dehydrogenase (ADH) or other oxidoreductases are responsible for the accumulation of benzyl alcohol and 1-phenyl-1,2-propanediol (PAC-diol) as unwanted by-products. In this work, a genome-scale metabolic model and flux balance analysis were used for identifying new target genes to improvement this biotransformation. The effect of gene deletions on the flux distributions in the metabolic model of S. cerevisiae was assessed using OptGene and minimization of metabolic adjustments (MOMA). Six single gene deletion strains, Δrpe1, Δpda1, Δadh3, Δadh1, Δzwf1 and Δpdc1, were predicted in-silico and further tested in-vivo by using knock-

out strains cultivated semi-anaerobically on glucose and benzaldehyde as substrates. For all strains, except Δadh1, higher amounts of L-PAC were produced as compared to the corresponding wild-type cells. Δzwf1 stands for the highest L-PAC formation (2.48 g/l) by 2 g/l of benzaldehyde which is 88 % of the theoretical yield. Findings of the present study can provide guidance for future works on the strain improvement to increase PAC production and demonstrate the successful utilization of computationally guided genetic manipulation to increase metabolic capacity.

Keywords: *Flux balance analysis, Genome-scale modeling, Metabolic engineering, L-Phenylacetylcarbinol, Saccharomyces cerevisiae*

[PS14-16] Environmental long-term Adaptation of Pichia pastoris – Universal Strategies and species-specific Traits of an industrial Yeast Species.

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Experimental evolution has been primarily applied in classical model organisms such as Escherichia coli and Saccharomyces cerevisiae. Thus, whereas the benefit of this approach has been evaluated for biotechnological applications of these species, little is known for non-conventional but biotechnologically important yeast species. Towards this end, we established a first comprehensive adaptive landscape of the methylotrophic yeast Pichia pastoris, which is applied in recombinant protein production processes. Environmental adaptation was performed by serial transfers in various growth environments and for several hundred generations, followed by growth profiling and genome sequencing. Several nutrient-rich and nutrient-poor growth environments with glucose and/or methanol as carbon sources were used for the adaptation of wildtype and glycosylation-deficient OCH1 knockout populations. Generally, adaptation was obvious as evolved populations showed increased growth rates or improved competitive fitness in adaptive conditions, whereas varying degrees of trade-offs were observed in non-evolutionary growth conditions. On the genome level, several environment-specific recurring mutational hotspots were observed among 55 evolved P. pastoris clones. For instance, multiple clones from independently methanol-evolved populations showed mutations of the alcohol oxidase 1 (AOX1) gene, leading to reduced AOX activity despite increased growth rates. Furthermore, methanol- and glucose-selection lead to multiple independent clones with mutations of two hitherto uncharacterized P. pastoris-specific transcription factors involved in environmental control. Another major mutational target was identified in the high osmolarity glycerol (HOG) signaling pathway. Strikingly, we observed HOG signaling pathway mutations in a genotype- and environment dependent manner as they emerged in opposing environmental conditions in wildtype and OCH1 populations. In this context, our data indicated a generally reduced adaptive potential, as well as diverging adaptive trajectories of glycosylation-deficient P. pastoris populations. Altogether, we provide evidence for highly

species-and genotype-specific mutations, which improves our understanding of the regulatory features of wildtype and mutant strains of biotechnologically relevant yeast species and also highlight the potential of this experimental approach for recombinant protein production processes.

Keywords: *Pichia pastoris, environmental stress, OCH1, experimental evolution, genome sequencing*

[PS14-17] High Resolution QTL Mapping of Ethanol Tolerance in Saccharomyces cerevisiae Using Advanced Intercross Lines (AIL) and Selective DNA Pooling (SDP)

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Ethanol, the end product of fermentation in Saccharomyces cerevisiae, is the main biofuel used worldwide. Since ethanol is toxic to the yeast cell, ethanol production is inhibited by its accumulation. Therefore, understanding the genetic basis of ethanol tolerance is important for the development of improved yeast strains with higher ethanol tolerance and yields. However, up to now, genomic elements affecting ethanol tolerance have only been mapped at low resolution, hindering their identification.

Here, we used Advanced Intercrossed Line (AIL) design, to perform high resolution mapping of QTLs affecting ethanol tolerance in yeast.

Selective DNA Pooling (SDP) and whole-genome sequencing were used in F6 of a cross between two widely separated S. cerevisiae haploid strains. Fifty and 95 QTLs affecting growth and survival, respectively, were identified by applying uniquely developed statistical methods.

The median size of a QTL Region (QTLR) was 12.1 Kb (growth) and 10.5 Kb (survival). These QTLRs are much narrower than previous reports. Importantly, some QTLRs included only a single gene. The mapping revealed significantly enriched biological processes important for ethanol tolerance in S. cerevisiae.

Keywords: *Ethanol tolerance, QTL mapping, Saccharomyces cerevisiae*

[PS14-18] Saccharomyces Cerevisiae as a Production Platform for Short-/Medium-chain Fatty Acid-derived Alcohols and Alka(e)nes

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Due to the limited nature of fossil fuel resources the demand for renewable bio-based chemicals and advanced biofuels will increase steadily. Fatty acids are considered as strategically important platform compounds and can suit as precursors for biofuels. Since mainly the chain length of hydrocarbons determines their physico-chemical properties

regarding combustion processes in engines, this is an important parameter. Here we will present the engineering of yeast for the production of specific short-/medium-chain fatty acid derivatives (C6-C12), such as fatty alcohols and fatty alka(e)nes which share substantial characteristics with currently used fossil fuels and give access to new drop-in biofuels without the need of engine or infrastructure adaption.

The yeast S. cerevisiae does not naturally accumulate short-/medium chain fatty acids. Therefore, metabolic engineering of the fatty acid synthetase (FAS) via rational mutagenesis to specifically produce short-chain fatty acids was done. Mutating one specific amino acid in the FAS complex (R1834K) enables S. cerevisiae to produce a high yield of octanoic acid (Nat. Commun. doi:10.1038/NCOMMS14650), a fatty acid with a length of eight carbon atoms. Based on octanoic acid it is necessary to engineer synthetic pathways specifically for the biosynthesis of short-/medium-chain fatty alcohols and alka(e)nes. One route is the conversion of octanoic acid to its corresponding aldehyde by the carboxylic acid reductase (CAR) from Mycobacterium marinum. This fatty aldehyde can then be reduced to 1-octanol by endogenous alcohol dehydrogenases (ADHs). 1-octanol has been identified as an attractive target compound with diesel-like properties. A big challenge for engineering such pathways is to achieve high yields and titers due to the low enzymatic activity of most enzymes, the toxicity of some target compounds, secretion of intermediates or production of undesired byproducts. Here, we will present the synthesis of 1-octanol with yeast and strategies to enhance its production.

Keywords: *Biofuel, Fatty acid biosynthesis, Fatty alcohol, Fatty alkanes/alkenes, Saccharomyces cerevisiae*

[PS14-19] Biodiversity-based Sources for Industrial Yeast Strains

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Industrial yeast strains require a set of traits – from altered metabolic fluxes to increased robustness – that are most often polygenic and as such rather difficult to engineer on the genomic level. Recent development of novel tools for analysis of polygenic traits in Saccharomyces cerevisiae and genetic characterization of a large number of its natural strains made it possible to relatively easily identify causal genes or alleles for biotechnologically relevant traits. These can then be transferred from strains possessing such traits to industrial strains which do not. We have applied such an approach to determine the genetic architecture of neutral lipid accumulation in S. cerevisiae and have been investigating the effect of the transfer of causal alleles between strains with initially different neutral lipid content. Our results show that, also for this non-selectable trait, the approach can result in accurate identification of causal alleles and in predictable effects of genetic manipulations of potential industrial yeast strains.

Apart from the within-species genetic diversity, also genes from even other domains of life can be very useful in the development of industrial yeast strains. One such example is utilization of genes encoding bacterial glycosidases for yeast strains developed for consolidated bioprocessing. To functionally assess potentially useful new enzymes, we have combined with yeast display a metagenomics approach that identified genes for glycosidases from ruminal bacteria evolved for specific biomass sources. Both approaches for tapping biodiversity-based sources discussed here are complementary and should enable advancement of development of new industrial yeast strains.

Keywords: *industrial yeast strains, polygenic traits, biodiversity, consolidated bioprocessing*

[PS14-20] A New Approach Based on the Function of Rho GTPase to Improve Ethanol Tolerance of Yeast Cells

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Ethanol tolerance, as a key feture for industrial yeast cells in ethanol production, is difficult to improve for the large amount of genes associated[1], thus many "irrational metabolic methods" such as gTME were employed[2]. It has been widely accepted that the cellular polarization change in Saccharomyces cerevisiae under ethanol stress is a kind of passive response and in turn causes deterioration of cell viability. However, we found a positive correlation between the ratio of elongated cells and ethanol tolerance, giving a new approach to improve ethanol tolerance of yeast cells via morpology remodeling. Rho GTPases in yeasts contribute directly to cellular polarization associated with the actin cytoskeleton[3], so we focus on the genes encoding Rho GTPase and the targets of their products.

Error-prone PCR was performed for the construciton of the libraries of random mutants of three Rho GTPases in the industrial yeast, followed by screening the mutants via ethanol stress. More than 50 mutants with significant improved ethanol tolerance were discovered, while with impared growth rate in some mutants. The work penetrated into ethanol tolerance research from a new perspective, providing a potential novel central and global regulation target.

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Keywords: *Ethanolo tolerance, Rho GTPase, Error-prone PCR*

[PS14-21] The α-factor peptide of high and stable activity via improved synthesis and purification.

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α1-Mating factor (in short, α-factor, WHWLQLKPGQPMY) is a 13-residue peptidic pheromone of Saccharomyces cerevisiae yeast, excreted from α-mating-type cells. It binds to G-protein coupled receptors (GPCRs) located in the membrane of mating type a cells, causing an increase of the transcription of genes required for mating, synchronization (a mitotic cell division cycle arrest in the G1 phase), and preparation of the cell for fusion with the opposite sex mating partner. Moreover, the binding of α-factor to its receptor induces the recovery of the cell from the G1 arrest by internalization of the receptor (probably alongside with the α-factor molecule).

As such, α-factor is a common reagent used in laboratory practice to synchronize yeast cultures for further experiments. Multiple reports from collaborating groups turned our attention to poor reliability of α-factor preparations coming from commercial sources and prompted us to redesign synthesis and purification of this technically demanding and very poorly soluble peptide. We developed improved synthesis method to obtain a product that is reproducibly more active than other commercially available peptides. We performed a simple experiment with Saccharomyces cerevisiae yeast (strain BY4147) to prove biological activity of our peptide. The yeast strain were put to grow and then arrested with different α-factors for 3 hours. After α-factor release, samples were collected and prepared for fluorescence-activated cell sorting (FACS) analysis. The flow cytometry results show that the Bal's Lab (our) α-factor synchronize the BY4147 strain at a concentration of 2 μg/ml. Two other commercial α-factors were unable to synchronize the BY4147 strain at this concentration. Further investigations indicated that at higher peptide concentrations, where all peptides were active, our α-factor acted faster than the commercial α-factors. Further research is underway to identify key differences in the compared preparations, including the impact of synthetic impurities on their activity.

Keywords: *α-factor, peptide synthesis, cell cycle, Saccharomyces cerevisiae, flow cytometry*

[PS14-22] 2-butanol production and tolerance in Saccharomyces cerevisiae

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The aim of this project is, first of all, to delete the ethanol production pathway and replace this with a production route for 2-butanol in S. cerevisiae. This will be done by:

• Changing kinetics and substrate specificity of a B12 independent bacterial diol dehydratase that is already expressed in S. cerevisiae and proven to be active with propanediol as a substrate.

• Using genetic engineering and redox constraints to minimize ethanol production and re-directing metabolic fluxes towards butanol production.

Secondly, to identify and utilize mechanism(s) underlying an increased tolerance to stress in general and to 2-butanol in particular. These investigations will include not only identification of specific proteins but also of specific mutations/alleles conferring increased tolerance.

So far, we have managed to establish 2-butanol production in S. cerevisiae by expressing a B12 dependent diol dehydratase together with a secondary alcohol dehydrogenase in this host. Furthermore, a butanol tolerant mutant of S. cerevisiae was evolved by an evolutionary engineering approach using repeated batch cultures of increasing 2-butanol concentrations. Proteomic analysis of the tolerant mutant compared to the wild type shoved that only 34 proteins were up-regulated of which 21 were constituents of the mitochondria. However, whole genome sequencing of the strains revealed a massive shift from mostly heterozygous tetraploid wild type, to a mostly heterozygous triploid mutant.

Keywords: *2-butanol, Metabolic engineering, Cell factory, Saccharomyces cerevisiae, Biofuel, Stress tolerance*

[PS14-23] Development Of Saccharomyces cerevisiae Screening Strain For Xylose Metabolism Application Through Evolutionary Engineering

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Saccharomyces cerevisiae is one of the most effective and the most used organism in the production of bioethanol. This well known yeast has its genetics, physiology and metabolic pathways well described and has been engineered for better application in industry for centuries. However, some challenges are still currently presented concerning S. cerevisiae application in bioethanol production, such as its inability of fermenting xylose. Second most abundant sugar present in lignocellulose biomass, xylose-fermenting yeast could enhance biomass utilization for ethanol production significantly.

This work presents the combination of genetic and evolutionary engineering strategies to develop xylose fermenting S. cerevisiae strains, and a screening strain adapted to xylose metabolism that was further utilized for the screening of new xylose isomerase.

Laboratory S. cerevisiae strains were constructed by overexpressing genes coding for xylose isomerase (XI) from Piromyces sp. and endogenous xylulokinase (XK) in pairs or individually. One of the strains (L7XIXK) harboring both XI and XK enzymes had higher fermentation rates than the others under aerobic fermentations, as expected. All generated strains underwent an evolutionary adaptive process in selected medium as sole carbon source. At the end, conditioned strains presented shorter lag growth phase and increased growth rate, increased xylose consumption rate (1.8 to 18.5 fold) and ethanol yield (up to 47% for L7XIXK) compared to each parental strain. The conditioned L7XIXK strain was cured, and acquired characteristics related to adaptation to xylose metabolism were maintained when transformed again with initial plasmids. Eight new genes coding for xylose isomerase were prospected, synthetized and further transformed into the curated strains. XI from Piromyces sp was used as control gene. Transformants were isolated and characterized through aerobic fermentations in minimal medium with xylose as only carbon source. Analysis of the fermentations performance of the transformants harboring new genes revealed a better performance of the strain transformed with the Piromyces gene.

Keywords: *Xylose isomerase, Saccharomyces cerevisiae, Evolutionary engineering, Ethanol, Biofuels, Xylose*

[PS14-24] Engineering Saccharomyces cerevisiae for the Production of Octanoic Acid

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Short-/medium chain fatty acids (SCFAs), such as octanoic acid (C8), have a wide range of applications in the cosmetic and nutraceutical sector but are also valuable precursors for biofuels. To produce SCFAs biotechnologically, the fatty acid metabolism of S. cerevisiae was redirected from the synthesis of long chain fatty acids – mainly C16/C18 chain lengths – to the production of SCFAs. By rational engineering of the endogenous fatty acid synthase (FAS), the production of SCFAs, mainly C6 and C8 fatty acids, could be achieved. By mutagenesis of up to five amino acids of the FAS gene, titers of 464 mg/L extracellular SCFAs and 245 mg/L octanoic acid were reached [Nat. Commun. doi:10.1038/ncomms14650].

Another interesting aspect is the release of the synthesized SCFA-CoA esters as free fatty acids from the yeast cell. Three putative thioesterases, which were suspected to be responsible for the release of the CoA-bound fatty acids, were analyzed. It could be shown that by deletion of EHT1, EEB1 and MGL2 the secretion of free SCFAs was almost completely abolished. Furthermore, the data suggests that Eht1p has a selective hydrolytic activity for octanoic acid-CoA esters, which makes this enzyme a promising candidate for further studies on octanoic acid secretion.

Here, we will show approaches for further SCFA yield increases as well as possible screening systems of newly engineered strains. Apart from this, a main obstacle that has to

be overcome is the toxicity of the compound. Strategies for generating strains with higher octanoic acid tolerance will be presented.

Keywords: *Short Chain Fatty Acids, Fatty Acid Synthase Engineering, Octanoic Acid/Caprylic Acid*

[PS14-25] Influence of culture media used for biomass production of Vishniacozyma victoriae on the biocontrol activity on pear postharvest diseases

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Vishniacozyma victoriae NPCC 1263 yeast biomass, with antagonistic properties against fungal pathogens of postharvest pear fruits, was produced in a previous work using a not optimized molasses-based medium (NOM). The objective of this work was to obtain an optimized a medium (OM) for biomass production in a large-scale bioreactor and to compare the in situ effectiveness of the control agent obtained from the two preparations at commercial scale (organic packinghouse).

Experimental Design using a molasses-based medium was employed for the optimization. Variables including molasses, urea, tiamine, KH2PO4 and ZnSO4 concentration as well as temperature were identified to show significant influence on the biomass production, and they were used to perform a Central Composite Experimental Design. According to these results, the highest biomass production was obtained with an optimize medium (OM) containing: 9% v/v molasses, 0.25% p/v KH2PO4, 0.25% p/v ZnSO4 ,0.25 ppm Tiamine and 1 g/l urea, at 13°C. Large scale biomass productions were carried out with both NOM (12.8% v/v molasses, 1 g/L urea, 20°C) and OM in a bioreactor (20L). Growth curves obtained in the bioreactor were fitted using the Gompertz model modified and kinetic parameters A and μmax were obtained (3.1 and 0.12 h-1 in OM medium and 2.6 and 0.04 h-1 in NOM medium, respectively) evidencing an improvement in culture parameters with the OM. Both cell viability following exposure of doses of H2O2 and intracellular ROS production were evaluated in yeast obtained from two preparations. The highest percentages of viability (60%) and the highest production ROS (33%) were obtained in cells from NOM compared with those cells from OM (30 and 3%, respectively).Finally, the biocontrol capacity of the two biomass preparations was evaluated in commercial conditions line-spray application with yeast (107 UFC/mL) in Packham's pear (1000 fruit).Treated fruits were stored at 1°C, and grey and blue mold disease incidence was recorded after 6 months of storage.

In efficacy trials on healthy pear fruit, C. victoriae grown in NOM effectively reduced incidence of Botrytis cinerea and Penicillium expansum in 66% and 86%, respectively while the same yeast grown in OM showed incidence reduction percentages of 63% and 26%, respectively.In conclusion, our findings suggest that resistance to oxidative stress could represent a mechanism by which V. victoriae regulates their viability and biocontrol efficacy when develops in NOM.

Keywords: *biocontrol, yeast biomass, stress oxidative*

[PS14-26] Surface Display of Recombinant Xylose Reductase by C-terminal or N-terminal Immobilization in the Saccharomyces cerevisiae

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Most of the constructs prepared so far for the surface display of recombinant proteins in yeast have been created with ubiquitous plasmids using the C-terminal part of α-agglutinin as an anchoring domain for recombinant protein. We have constructed two genetic cassettes for the surface display of heterologous proteins in S. cerevisiae, consisting of a strong and regulated host promoter GAL1, a signal sequence for directing the protein into the secretory pathway, an anchoring domain of native cell wall proteins for C- or N-terminal immobilisation, and genetic tags for easy detection of the recombinant protein. YEp351Pir4 plasmid was constructed for the N-terminal immobilisation of heterologous proteins, containing PIR4 under a GAL1 promoter followed by the spacer region (a stretch of eight serine residues), a region consisting of several restriction sites for the insertion of the gene of interest and, finally, followed by the -6xHis and -HA tags. The plasmid pRS425Ccw12 was prepared for C-terminal immobilisation of heterologous proteins. This plasmid contains a GAL1 promoter followed by the part of CCW12 which is coding for the signal sequence, the -HA tag, restriction sites for the insertion of the gene of interest, the part of the CCW12 which is coding for the GPI anchoring signal, and the downstream genetic elements of the CCW12. The S. cerevisiae gene GRE3 coding for intracellular xylose reductase (XR) was inserted into the plasmids described above using suitable restriction sites. Finally, the construct pRS425Ccw12XR was modified by introducing the STOP codon immediately after GRE3 coding region, resulting in a secretion of recombinant xylose reductase into the growth medium. Finally, the localization, relative abundance, activity and characteristics of different forms of recombinant xylose reductase were determined. The results obtained were compared to find out which type of immobilization is the best for the surface display of this particular enzyme.

Keywords: *surface display, xylose reductase, recombinant protein*

[PS14-27] Compatibility of Industrial Saccharomyces uvarum and Saccharomyces cerevisiae with Oenococcus oeni During Synthetic Red Juice Fermentation

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Wine yeast and lactic acid bacteria (LAB) are fundamental to the success of wine fermentation. Recognition of compatible yeast and LAB strains for co-inoculation during red wine production is becoming increasingly important for the robustness of winemaking on a large scale. This study aimed to identify industrially relevant yeast and LAB pairs that are able to complete both alcoholic (AF) and malolactic fermentation (MLF) when co-inoculated in red chemically defined grape juice medium (RCDGJM). The compatibility of three commercially available wine yeasts (Velluto BMV58, Saccharomyces uvarum; Lalvin

ICV GRE, S. cerevisiae; NT50, S. cerevisiae hybrid) and six commercially available LAB (O-MEGA, PN4, Lactoenos SB3 Direct, Viniflora CH16, Lactoenos 450 PreAc, Lalvin VP41; all Oenococcus oeni) were investigated in RCDGJM fermentations. AF and MLF were analysed by sugar and L-malic acid enzymatic assays, and performance statistically analysed using area under the curve analysis. Although AF by the yeast was unaffected by co-inoculation with any of the LAB, MLF efficiency of the O. oeni strains were affected by the yeast. This effect was only partly accounted for by a rapid increase in ethanol concentration. S. cerevisiae hybrid NT50 and S. cerevisiae strain Lalvin ICV GRE completed alcoholic fermentation in 5 and 8 days, respectively. Although NT50 finished AF before Lalvin ICV GRE, and subsequently would have made the ethanol concentration increase faster, it was compatible with three LAB, whereas Lalvin ICV GRE was only compatible with one. S. uvarum strain Velluto BMV58 took an average of 13 days to complete alcoholic fermentation and subsequently was compatible with four of the six LAB. However, none of the six LAB tested were able to complete MLF with all three yeast, indicating that compatibility is highly strain dependant.

Keywords: *Saccharomyces cerevisiae, Winemaking, Co-inoculation*

[PS14-28] FUNCTIONAL OVEREXPRESSION OF GENES INVOLVED IN ERYTHRITOL SYNTHESIS IN THE YEAST YARROWIA LIPOLYTICA

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Erythritol, a four-carbon polyol synthesized by microorganisms as an osmoprotectant, is a natural sweetener produced on an industrial scale for decades. Despite the fact that the yeast Yarrowia lipolytica has been reported since the 1970s as an erythritol producer, the metabolic pathway of this polyol has never been characterized. It was shown that erythritol synthesis in yeast occurs via the pentose phosphate pathway (PPP). The oleaginous yeast Y. lipolytica is a good host for converting inexpensive glycerol into a value-added product such as erythritol. Glycerol is a renewable feedstock which is produced on a large scale as a waste product by many branches of industry.

In this light, we functionally overexpressed four genes involved in the pentose phosphate pathway (PPP): gene YALI0E06479g encoding transketolase (TKL1), gene YALI0F15587g encoding transaldolase (TAL1), gene YALI0E22649g encoding glucose-6-phosphate dehydrogenase (ZWF1), and gene YALI0B15598g encoding 6-phosphogluconate dehydrogenase (GND1). Here, we show that the crucial gene for erythritol synthesis in Y. lipolytica is transketolase. Overexpression of this gene results in a 2-fold improvement in erythritol synthesis during a shake-flask experiment (58 g/L). Moreover, overexpression of TKL1 allows for efficient production of erythritol independently from the supplied dissolved oxygen. Fermentation conducted in a 5-L bioreactor at low agitation results in almost 70% higher titer of erythritol over the control strain.

This work presents the importance of the PPP in erythritol synthesis and the possibility for economical production of erythritol from glycerol by the yeast Y. lipolytica.

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Keywords: *Yarrowia lipolytica, erythritol, glycerol, metabolic engineering*

[PS14-29] Characterization of erythrose reductase from Yarrowia lipolytica and its influence on erythritol synthesis

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Yarrowia lipolytica is a well-studied nonconventional yeast that can produce erythritol as a osmoprotectant. This four-carbon polyol is widely used in the food industry as a sweetener. It was shown that erythritol synthesis in yeast occurs via the pentose phosphate pathway (PPP)[1]. Erythrose reductase (ER) plays a significant role in synthesis of erythirtol. ER catalases the final step of the process which is reduction erythrose to erythritol with NAD(P)H as a cofactor. In Y. lipolytica important role in erythritol synthesis plays the aldo-keto reductase (AKR) superfamily consisting of eight proteins. The amino acid sequence of the YALI0B07117p protein showed a high homology (38%) to the previously described erythrose reductases from other known erythritol producers (Candia magnoliae). In this study we characterized and investigated the influence of the protein overexpression on the erythritol synthesis in Y. lipolytica.

This work was financed by the Polish National Centre for Research and Development under project LIDER/010/207/L-5/13/NCBR/2014.

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Keywords: *Yarrowia lipolytica, Erythrose reductase, Erythritol*

[PS14-30] Optimization of the Production of Recombinant HPV Proteins in Yeast for a Biosensor-Based HPV Detection Platform

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Human papillomaviruses (HPV) are DNA viruses that infect skin or mucosal epithelial cells. Among more than 100 types of HPV, there are over a dozen of genotypes recognized with a

potential to cause cervical cancer. Types 16 and 18 are the most dangerous, accounting for more than 70% of cancers. Although the great majority of infections resolve within two years, 13 phylogenetically-related HPV16 genotypes can cause both cervical and oropharyngeal cell squamous cell carcinoma (OPSCC) (1). The oncogenicity of HPV is mainly due to the activity of its early proteins E4, E6 and E7, which impair growth regulatory pathways. In particular E6 seropositivity is the best predictor of HPV16-driven OPSCC (2). However, the strong immunoresponse is against the major and minor capsid proteins (L1 and L2, respectively) that have been used so far to develop broad spectrum-vaccines (3).

In order to construct a biosensor for the quick and easy detection of HPVs antibodies, we amplified early and late proteins of HPV16 from a patient (ISS, Rome) and cloned them into an inducible expression yeast vector named pBLAST previously developed (4). This 2 micron-derived vector is characterized by a high copy number, ensuing a FRT/Flp site-specific recombination, and by a MF alpha 1 signal sequence to extrude the final protein. Combining this episomal plasmid with an ad hoc optimized protease-deficient yeast host strain, we developed a portable system to produce HPVs peptides with good efficiency and low toxicity in Saccharomyces cerevisiae. Using this system, we aim to produce proteins from ten out of 13 “High-Risk” types of HPVs. These recombinant and purified proteins will be coated on our carbon nanotubes layer electrodes in order to develop a fast and affordable HPV immunosensor based on impedance detection.

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Keywords: *S. cerevisiae, HPV, biosensor*

[PS14-31] Development of high efficient systems for producing functional cosmetic peptides in the yeast

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The growth of global cosmetic industry has been increasing fast because of strong lasting interests in beauty and well-being age. Among the cosmetic products, skin and hair-care have the highest market share up to 70%. Recently, functional peptides have been widely used as a component in skin and hair-care cosmetic products. Some famous peptides play

roles in activation of collagen and wrinkle improvement. However, most of these peptides have been manufactured by chemical synthesis, resulting in high cost and existent of toxic chemicals. Therefore, these problems can be overcome by biological synthesis using GRAS yeast.

In this study, we developed biological systems to produce and purify these functional peptides in the GRAS yeast, Pichia pastoris. First, we constructed an expression vector (pBJYpep) in which each peptide fused to His-tag could be expressed and secreted into media broth. These vectors were transformed into P. pastoris individually and each recombinant strain was cultivated in minimal media (YNB/YSD without adenine). We used an affinity column (Ni-column) to purify each expressed peptide and treated the expressed peptide with specific protease to remove the His-tag. We confirmed the expressed and analyzed each peptide on SDS-PAGE and HPLC/ESI-MS, respectively. We are developing optimized condition to increase concentration of the expressed peptides and economic values by optimization of culture media, fermentation mode and purification process. These systems will be applied to widely in production of cosmetic, cosmeceutical and pharmaceutical peptide.

Keywords: *Yeast, Peptide, Pichia pastoris, pharmaceutical*

[PS14-32] Deletion of a Novel Mitochondrial Carrier for Citrate Shifts Acid Production Towards Isocitric Acid in Yarrowia lipolytica

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Background: The hemiascomycetous yeast Yarrowia lipolytica is capable of producing high amounts of various organic acids, like citric acid (CA) and isocitric acid (ICA). ICA is an increasingly important building block chemical for industrial, dietary or pharmaceutical applications. The production of ICA in Y. lipolytica is accompanied by a significant amount of CA, which reduces the yield of the desired product. Focusing on this oleaginous yeast as metabolic chassis offers a strategy to achieve commercially viable yields for ICA production.

Results: In this study, we identified and deleted a gene for the putative mitochondrial citrate and oxoglutarate carrier YHM2 in order to prevent the export and secretion of CA and to increase the production of ICA. The deletion of the YHM2 gene resulted in a strong shift in ICA/CA product ratio from 12 % to 90 % compared to the wild-type strain. However, the total amount of produced acids (CA & ICA) was decreased from 59.8 g l-1 to 10.5 g l-1. Under production conditions in a 600-ml fermenter, the YHM2 deletion strain produced 15.5 g l-1 ICA (ICA/CA ratio: 95 %) after 5 days with glucose as carbon source and 71.3 g l-1 ICA (ICA/CA ratio: 86 %) with sunflower oil as the carbon source.

Single-copy overexpression of YHM2 resulted in a slight increase in the ICA/CA product ratio to 22 % with also decreasing the overall acid production.

Conclusion: The mitochondrial CA transporter YHM2, which has been identified within this work, represents an interesting metabolic target for the production of isocitric acid with the yeast Y. lipolytica. However, further work has to be done in order to reach a commercially viable productivity and purity of ICA.

Keywords: *Citrate Transporter, YHM2, Isocitric acid, Citric acid, Yarrowia lipolytica*

[PS14-33] Novel Antifungal Peptide Secreted by Candida intermedia LAMAP1790 Active Against Brettanomyces bruxellensis, a Wine Spoilage Yeast

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Brettanomyces bruxellensis has been described as a principal contaminant of the wine industry. To avoid its growth, the must is commonly supplemented with SO2, one of the most used preservatives in the food industry. However, the use of this compound has been questioned for the potential health problems that entails its consumption. With the aim of exploring other alternatives, our laboratory has previously described a strain Candida intermedia LAMAP1790 as a productor of antimicrobial compounds. Thus, the objective of this work was to determine the antifungal activity of the compound produced by C. intermedia against B. bruxellensis and elucidate its chemical nature. Using joint seeding tests and viability after direct exposure to sterile culture medium of C. intermedia LAMAP1790, the secreted compound was found to have fungicidal activity on four strains of B. bruxellensis, without affecting the growth of Saccharomyces cerevisiae. Posteriorly, in order to determine the chemical nature of the compound, the proteins in the supernatant were fractionated and concentrated 250X, which revealed that the antifungal activity is related to the presence of two peptides with a molecular mass less than 5 kDa. Using 2D-nano-LC-MS/MS we reported the sequence of a 33 residues cationic peptide present in the culture media, whose molecular mass was 3.2 kDa and an isoelectric point of 9.72. Finally, using a bioinformatics approaches, it was determined that the peptide present homology to a transmembrane segment of yeast MIP aquaporine superfamily and the 3D structure corresponds to a positively charged amino terminal segment followed by an α-hélix structure that stabilize the hydrophobic residues, allowed its amphiphilicity. These results allow to establish that the secreted peptide have biochemical and structural similarities to antimicrobial peptides (AMPs) as the ones described in filamentous fungi, with possible biotechnological applications in the control of spoilage microorganisms in the wine industry.

Keywords: *Wine Microbiology, Biocontrol, Antifungal Peptides*

[PS14-34] Elucidation of the Mechanism Underlying an Adaptive Evolution to Improve in a Capacity to Metabolize 4-Deoxy-L-erythro-5-hexoseulose Uronate (DEH) for Alginate Utilization in Engineered Saccharomyces cerevisiae

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Japan is surrounded by a vast sea area under its jurisdiction that provides an immense opportunity for utilizing marine resources to improve the quality of life. Our goal is to produce valuable, safe, cost effective, and sustainable fuels and chemicals using microbes that utilize marine sugars, including alginate and mannitol derived from marine brown macroalgae. Moreover, we have focused on the budding yeast Saccharomyces cerevisiae, a safe and productive industrial microbe that has been widely used as a microbial cell factory.

However, this budding yeast is unable to utilize either alginate or D-mannitol. Alginate can be depolymerized by both endo-type and exo-type alginate lyases, yielding a monouronate, 4-deoxy-L-erythro-5-hexoseulose uronate (DEH), a key intermediate in the metabolism of alginate. In this research, we constructed engineered two S. cerevisiae strains that are able to utilize both DEH and D-mannitol on two different strain backgrounds (BY4742 and D452-2 strains), and we also improved their aerobic growth in a liquid medium containing DEH through adaptive evolution (–160 generations, –30 times subcultures) (1). In both evolved strains, one of the causal mutations was surprisingly identical, a c.50A>G mutation in the codon-optimized NAD(P)H-dependent DEH reductase gene, one of the 4 genes introduced to confer the capacity to utilize DEH. This mutation resulted in an E17G substitution at a loop structure near the coenzyme-binding site of this reductase, and enhanced the reductase activity as well as aerobic growth in both evolved strains (1).

Thus, the crucial role for this reductase reaction in the metabolism of DEH in the engineered S. cerevisiae is demonstrated. This finding provides significant information for synthetic construction of a S. cerevisiae strain as a platform for alginate utilization.

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Keywords: *brown macroalgae, alginate, mannitol, 4-deoxy-L-erythro-5-hexoseulose uronate, DEH, DEH reductase, adaptive evolution*

[PS14-35] Remediation of Groundwater: MTBE Degradation by Recombinant Yeast Cells Expressing CYP2B1-Variants.

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The BMBF-funded "Innovativer regionaler Wachstumskern" BIOSAM with 11 companies and 6 research institutions addresses the potential of whole cells as sensors and actors for applications in biotechnology, environmental and medical technology. The BIONews project of BIOSAM aims to evaluate the capacity of yeast cells to act as actors in water treatment in order to degrade various pollutants of water. We investigate the possibility of genetically modified yeast cells in order to remove MTBE from groundwater. As a widely used fuel additive, MTBE is an important groundwater contaminant that is hardly degradable and discussed to possess a cancerogenic potential.

Keywords: *Saccharomyces cerevisiae, MTBE, CYT P450, CYP2B1*

[PS14-36] Sustainable Production of Health and Nutrition Ingredients Using Baker's Yeast

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Nature contains a wide range of small molecules that can be used as health and nutrition ingredients. Most of these molecules are made from fossil fuels, plant extracts, or other natural materials. While the issue of using petrochemicals for manufacturing is obvious, much less appreciated is the fact that using plant extracts or other natural materials may also be unsustainable and lead to the extinction of natural resources. Therefore, a more environmentally friendly and sustainable production method needs to be developed to cope with the growing demand for natural health and nutrition ingredients.

Evolva is the leading company in developing fermentation-based methods of producing health and nutrition ingredients – solving nature's supply chain issues. Our state-of-the-art knowledge, technology and methodology allow us to develop biosynthetic routes to produce valuable small molecules by genetically engineering the metabolic pathways in Saccharomyces cerevisiae, baker's yeast. Manufacturing by fermentation makes the production process environmentally sustainable and significantly reduces production costs. Up to now, Evolva has successfully introduced several natural molecules to the market, including vanillin, resveratrol, nootkatone, and valencene, that have broad applications in nutrition, personal care and flavours and fragrances. The natural zero-calorie, high-intensity stevia-sweetener EverSweet is scheduled to be launched in 2018. Furthermore, Evolva has developed a product pipeline in order to introduce more valuable natural molecules to our customers in the near future.

Keywords: *ingredient, fermentation, sustainable*

[PS14-37] Improvement of Acid Tolerance and Lactic Acid Production by Up-Regulation of Sterol Metabolism in Saccharomyces cerevisiae

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For the production of lactic acid as a precursor of biodegradable polyester, polylactic acid (PLA), Saccharomyces cerevisiae was engineered to redirect the ethanol fermentation pathway to lactic acid by the replacement of pyruvate decarboxylase 1 (PDC1) gene to bacterial lactate dehydrogenase (LDH). Lactic acid production, however, was limited by inhibitory effects of acidic condition on cell growth by accumulated lactic acid in the media. Acid-tolerance of host strain is very important to minimize the use of neutralizing base (calcium hydroxide) and thus develop a gypsum-free process for lactic acid production. To improve the acid-tolerance of recombinant yeast, we explored genes related to acid-tolerance in Pichia kudriavezevii, which is one of the yeasts capable of growing at very low pH. It was discovered that a set of genes involved in ergosterol biosynthesis including the transcriptional regulator UPC2 was up-regulated. To increase the cellular supply of ergosterol, we introduced an extra-copy of the UPC2 gene into the lactic acid-producing S. cerevisiae. The engineered strain, possessing a highly active ergosterol biosynthetic pathway, showed an increased lactic acid tolerance and accumulated 50% more lactic acid than that of wild-type strain. This study demonstrates a novel approach that improves the lactic acid-tolerance by metabolic engineering of the ergosterol biosynthetic pathway and increases the level of lactic acid production in yeast.

Keywords: *Acid tolerance, Lactic acid, Genome-wide expression, UPC2, Sterol metabolism*

[PS14-38] Direct Fermentation of Jerusalem Artichoke Tuber Powder for Production of L-Lactic Acid and D-Lactic Acid by Metabolically Engineered Kluyveromyces marxianus

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A cost-effective production system for optically pure L- and D-lactic acid (LA) from Jerusalem artichoke tuber powder (JAP) was developed by metabolic engineering of Kluyveromyces marxianus. To construct LA-producing strains, the ethanol fermentation pathway of K. marxianus was redirected to LA production by disruption of KmPDC1 and expression of L- and D-lactate dehydrogenase (LDH) genes derived from Lactobacillus plantarum under the control of the K. marxianus translation elongation factor 1α promoter. To further increase the LA titer, the L-LA and D-LA consumption pathway of host strains was blocked by deletion of the oxidative LDH genes KmCYB2 and KmDLD1. The recombinant strains produced 130 g/L L-LA and 122 g/L D-LA by direct fermentation from 230 g/L JAP containing 140 g/L inulin, without pretreatment or nutrient supplementation. The conversion efficiency and optical purity were >95% and >99%, respectively. This production system using JAP and the inulin-assimilating yeast K. marxianus is suitable for cost-effective production of LA.

Keywords: *Jerusalem artichoke, Inulin, Kluyveromyces marxianus, Lactic acid, Lactate dehydrogenase*

[PS14-39] Production of Lipids by Yeast Yarrowia lipolytca on Seawater-based Medium

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To make a biotechnological production more economically available and widely used new technologies must be developed to reduce costs on energy consumption, freshwater and substrate usages. Usually bio-processes that require the growth of microorganisms in large volumes require enormous amounts of water. In bio-industry mostly freshwater is used, which cause the competition with the constantly decreasing access to clean and good quality freshwater.

For this reason, the possibility of intracellular lipids production by oleaginous yeast Yarrowia lipolytica in seawater-based medium was investigated. During the study, wild type strain A101 and recombinant strain AJD DGA1, overexpressing diacylglycerol acyltransferase - DGA1, the final step of the triglyceride (TAG) synthesis pathway, were grow in artificial seawater based medium (as a control distilled water medium was used). First, the growth of yeast was study in microplate reader BioscreenC on rich and minimal media, later in shake flask scale. In all experiments growth of yeast was on similar level for seawater-based medium and control medium. Next, the lipid production by yeast was investigated in seawater-based media containing different carbon sources: glucose, glycerol, mix of xylose+glucose, acetate and sucrose. Production of lipids were on comparable level for seawater salinity medium and control. Finally, the lipid production on media containing waste materials as a carbon source: crude glycerol and molasses, was investigated. The production of lipids on crude glycerol by strain AJD DGA1 in seawater salinity achieve 4.29 g/L (freshwater control 4.11 g/L), which represents 40% of dry cell weights. The obtained results have shown the possibility of growth and lipid production by yeast Y. lipolityica on seawater-based medium.

Keywords: *lipid production, seawater, Yarrowia lipolytica, waste materials*

[PS14-40] Modified strains of Saccharomyces cerevisiae used for monitoring of specific chemical substances

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The red/white colony colour assay utilizes the fact that ade2 and/or ade1 mutations in the adenine biosynthetic pathway (AMP pathway) lead to accumulation of AIR (P-ribosylaminoimidazole), the substrate of Ade2p. The AIR is oxidized to a red pigment, which causes a change of colony colour from white to red. This phenomenon has been widely

utilized, for example, to visualise various genetic events (recombination, aneuploidy), to study plasmid stability and to score for prion [PSI+] presence in yeasts.

We have developed a new application of this phenomenon. We have constructed ade2 strains with an additional modification of the AMP pathway by introducing an inducible promoter in front of the ADE5,7 gene (Czech patent CZ 305223) (1). This genome modification enables accumulation of the red pigment only when an appropriate inducer is present. Thus a strain modified by introducing the promoter PGAL1 (GAL1 promoter, inducible by galactose) in front of the ADE5,7 gene starts to produce red pigment when galactose is present in the growth medium (1) and a strain modified by introducing the promoter PCUP1 (CUP1 promoter, inducible by Cu2+ ions) produces pigment only in the presence of copper ions (2). Colonies or immobilized cells of these strains turn red after specific inductor treatment. The latter strain was successfully used as a biosensor for the determination of copper concentrations in real samples of water contaminated with copper ions (2). The system can be used for monitoring of other compounds depending on suitable promoter availability.

This work was supported by Technology Agency of the Czech Republic (TA01011461).

1. Czech patent CZ 305223

2. Vopalenska, I, Vachova, L, Palkova, Z. (2015) New biosensor for detection of copper ions in water based on immobilized genetically modified yeast cells. Biosensors and Bioelectronics 72:160-167

Keywords: *Yeast biosensor, Copper ion detection, Purine synthesis pathway, Contaminated water*

[PS14-41] Yeast Strain Characterization and Process Optimization

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Harnessing the metabolism of cells to enhance the production of native metabolites or novel compounds leads to a variety of potential production strains. In order to select the best performing strain efficient screening is required paired with thorough characterization and evaluation. Continuous cultivation is the method of choice for precise physiological characterization of microbial cell factories, whereas fed-batch cultivation is preferred for future industrial applications at large scale. Cultivation techniques and conditions comparable to the final production process are promising, since strain physiology is significantly contributing to the overall strain performance. However, screening libraries of potential production strains calls for parallel cultivations at small scale. In order to address these issues, various small-scale bioreactor systems have been developed with the purpose of i) granting flexibility with regard to substrate and forced

nutrient limitations, control of growth rates, cultivation conditions and of ii) allowing transferability to larger fermentation scales.

A system of 24 parallel bioreactors with online monitoring of dissolved oxygen for continuous cultivations and a fed-batch mimicking medium applicable in small-scale volumes for parallel fed-batch cultivations are explored for applications with Saccharomyces cerevisiae.

Keywords: *bioreactor, small-scale, fed-batch cultivation, continuous cultivation*

[PS14-42] Substrate specificity determinants in invertases and inulinases of sour dough yeasts and filamentous fungi

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We have sequenced the SUC/INU genes from genomic clones or from PCR products from Finnish rye bread sour dough yeasts C. milleri CBS 8195 and T. delbrueckii ABM2364; Kluyveromyces marxianus ABM 5130 and the type strains of C. milleri ATCC 96244, S. cerevisiae CBS 1171, and T. delbrueckii CBS 1146,. Sequences for K. marxianus, Schwanniomyces occidentalis, Aspergillus awamori, and Trichoderma harzianum were obtained from public databases (PDB, NCBI, EBI). C. milleri CBS 8195 had three almost identical SUC genes on three different chromosomes, thus, forming a polymorphic SUC family in this species, comparable to the S. cerevisiae SUC family.

The substrates, the disaccharide sucrose, (Glu α1->2βFru) and inulin, (Glu α1->α2 [Fru β2->α2 Fru]n n=2-60) are very similar in the basic structure. In some studies, the substrate specificity of β-fructofuranosidases has been investigated but the information is partial and scattered, and there is no specific study on this issue. The invertase / inulinase ratio, or S/I ratio, describes substrate specificity in a quantitative manner, a low value indicating an inulinase. We have found the S/I ratios to differ, and accordingly were put in a decreasing order from lowest to highest inulinase activity: Saccharomyces > Candida > Torulaspora > Kluyveromyces > Aspergillus ~ Trichoderma. This enabled us to analyze the multiple sequence alignment and the published structures and our modeled structures of the proteins in relation to the S/I ratio, and determine which amino acids influence the substrate specificity and to what extent.

Enzyme activities were assayed by measuring reducing power with DNS assay. Multiple sequence alignment (MSA) was done using MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/), structural modeling was done with Robetta (http://robetta.bakerlab.org/), and viewing in PyMol.

Keywords: *Candida milleri, Torulaspora delbrueckii, Invertase/inulinase, Substrate specificity determinants*

[PS14-43] Biotechnology Application of Fungal Laccases Produced by Saccharomyces cerevisiae

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Laccases belong to the group of oxidoreductases and are often called as “green catalyst”, because during the reaction, oxygen from the air is consumed and the only by-product is water without forming any toxic side products. Laccases can convert a number of different compounds, including phenols, aromatic and aliphatic amines. For these reasons, laccases have a great potential for a wide range of industrial and biotechnological application. The aim of this work is recombinant production of three laccases from different source of origin, characterization of their properties and application of these enzymes in processes related to decolorization of dyes and real wastewater from textile industry.

Laccase genes from i) Myceliophthora thermophila (MtL), ii) Trametes versicolor (TvL), and iii) Trametes trogii (TtL) were cloned into series of vectors by homologous recombination and successfully expressed in Saccharomyces cerevisiae. For an efficient production of active laccases, expression conditions were optimized and secreted laccases were isolated from the medium by consecutive hydrophobic and gel chromatography. Biochemical characterization of purified laccases showed differences in their stabilities, specific and substrate activities, and their abilities to decolorize tested samples.

Supported by NPU LO 1302 and TA CR grant TA0101 1461.

Keywords: *laccases, heterologous expression, biotechnology*

[PS14-44] Tuning the Sensitivity of the PDR5 Promoter-Based Detection of Diclofenac in Yeast Biosensors

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The commonly used drug diclofenac is an important environmental anthropogenic pollutant. Currently, detection of diclofenac is mainly based on chemical and physical methods. Here we describe a yeast biosensor that drives the diclofenac-dependent expression of a recombinant fluorescent protein from the authentic promoter of the PDR5 gene. This key component of the pleiotropic drug response encodes a multidrug transporter that is involved in cellular detoxification. We analyse the effects on diclofenac sensitivity of artificial PDR5 promoter derivatives in wild-type and various yeast mutant strains. This approach enabled us to generate sensor strains with elevated drug sensitivity.

Reference: Schuller A., Rödel G. and Ostermann K. (2017); Sensors 17(7):1506; doi:10.3390/s17071506

Keywords: *diclofenac, PDR5, yeast, biosensor*

[PS15] New tools in yeast research

[PS15-1] How To Observe Single Molecules Of Transcription Factors In Yeast Saccharomyces

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In vivo Single Molecule Tracking (SMT) has recently developed into a powerful technique for measuring and understanding the transient interactions of transcription factors (TF) with their chromatin response elements. The role that this rapid exchange plays in transcription remains hotly debated. Is this noise, or is it transcriptionally productive? This method was successfully applied to mammalian cells but in yeast it remains problematic. Key technical problems are (a) a lack of robust procedures to determine if the labeling strategies used to mark the transcription factor are innocuous and therefore to ensure that SMT estimates are correct; (b) low retention of the bright and stable organic dyes covalently binding to HaloTag used in the state-of-the art SMT experiments; (c) the small size of the yeast nucleus, which is ~10-fold smaller in diameter than mammalian cells, and therefore the imaged area is ~100-fold smaller. To facilitate the dye incorporation we disrupted ATP-binding cassette multidrug resistance (ABC-MDR) transporter PDR5. We developed new and robust procedure for evaluation of adverse effects of labeling, and new quantitative analysis procedures that significantly improve residence time measurements by accounting for fluorophore blinking. We estimated residence time of the yeast transcription factor Ace1p on non-specific and specific chromatin sites. Estimates of TF binding to specific sites clarify the function of the fast exchange and provide a deeper understanding of the molecular mechanisms of transcription initiation. Our results provide a framework for the reliable performance and analysis of SMT of transcription factors in yeast.

Keywords: *Single Molecule tracking, HaloTag, Fluorescence microscopy, Saccharomyces, Transcription Factors, CUP1, ACE1*

[PS15-2] The Daughter Extinction Program (DEP): a synthetic biology approach to explore the genetic and metabolic landscape of mitotic ageing

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Eukaryotic cells can only undergo a limited number of cells divisions, which defines the mitotic age of the cell. Although extensively investigated, high-throughput assays to assess mitotic ageing are still lacking. To address this limitation, we developed the Daughter Extinction Program (DEP) in the yeast Saccharomyces cerevisiae, which inducibly arrest cell cycle in daughter cells. Using two independent, daughter cell-specific, inducible

systems, we were able to selectively prohibit cell division in daughter cells of prototrophic, diploid yeast cells. As our DEP renders an exponential growing population to a linear function of dividing mother cells, measurements of cell densities allow us to determine median mitotic lifespan, thereby clearing the way for fully automated, high-throughput measurements. Using synthetic genetic array (SGA)-technology, we introduced our DEP into the existing yeast libraries (YKO and Damp) and for the moment, we are evaluating mitotic ageing. Moreover, our system is perfectly equipped to evaluate the effect of different metabolic conditions as well as chemical compounds on mitotic ageing, allowing for n-dimensional functional clustering of each gene and straightforward identification of compounds affecting mitotic ageing.

Keywords: *mitotic ageing, system-wide approach, new yeast research tool*

[PS15-3] High-Quality Yeast Genome Assembly And Efficient Structural Variant Detection Using Nanopore Sequencing

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Comprehensive genomic variant maps are essential to explore genome evolution as well as its phenotypic consequences in natural populations. To date, short-read sequencing allowed to have genome- and species-wide views of mainly single nucleotide and copy number variants as we recently obtained in the Saccharomyces cerevisiae species by whole genome sequencing of 1,011 natural (http://1002genomes.u-strasbg.fr/) isolates using an Illumina technology. However, the detection of structural variants (e.g. long indels, inversions, translocations) (SVs) still poses challenges, more precisely when variants are in high complexity regions while they correspond to genetic variants underlying phenotypic variation. Emerging long-read sequencing technologies, such as Oxford Nanopore MinION sequencing, provide an unprecedented opportunity to efficiently detect these structural variants. To evaluate the performance of this technology for whole-genome assembly and SVs detection, we resequenced various genomes of natural isolates of two distinct yeast species, namely Saccharomyces cerevisiae and Dekkera bruxellensis, showing different degree of genomic complexity. Using the ONT MinION at moderate coverage (~20x), highly complete and contiguous assemblies have been obtained. Data generated allowed hence to accurately detect SVs, such as translocations and large inversions throughout the genomes. Among the long inserted and deleted regions, we identified those related to transposable elements and could provide a complete cartography of these elements among the sequenced isolates. Our results clearly show the value of the MinION system for screening whole genomes for complex SVs and deeply characterizing genome architecture in yeast natural populations.

Keywords: *structural variant detection, Oxford Nanopore MinION sequencing, whole-genome assembly*

[PS15-4] AYD Drop-out: A Useful Medium for Various Yeasts

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Yeasts are usually grown in YPD or synthetic dextrose (SD) medium containing ammonium sulfate and yeast nitrogen base (YNB). To know the nutrients required for the best growth of yeasts, SD medium may not be suitable because yeast growth in SD is not comparable to YPD rich medium. We have developed AYD medium containing amino acids, potassium phosphate, magnesium sulfate, glucose, vitamins, and metals, which showed comparable growth to YPD. By using AYD, we have determined vitamins and metals required for the growth in various yeasts. Vitamins and metals contained in AYD are inositol, pantothenic acid, nicotinic acid, pyridoxine, thiamine, biotin, ferric chloride, and zinc sulfate. The yeast species tested were Saccharomyces cerevisiae (Sc), Saccharomyces pasteurianus (Sp), Kluyveromyces marxianus (Km), Kluyveromyces lactis (Kl), Candida utilis (Cu), Candida albicans (Ca), Candida glabrata (Cg), Scheffersomyces stipitis (Ss),and Yarrowia lipolytica (Yl). In AYD, all these strains grew similarly as in YPD. Therefore, other vitamins and metals contained in YNB, such as 4-aminobenzoic acid, riboflavin, boric acid, folic acid, copper sulfate, potassium iodide, manganese sulfate, sodium molybdate, sodium chloride, and calcium chloride are not required for growth in these yeasts. From AYD, each vitamin or metal was eliminated and the growth of yeasts was measured. All Sc strains including Japanese sake strain Kyokai No. 7, bio-ethanol strain, and laboratory strain S288C, and beer yeast Sp did not grow without pantothenic acid. Similarly, pantothenic acid and nicotinic acid were required in Kl and Km, nicotinic acid, pyridoxine, and thiamine were in Cg, and thiamine was in Cu and Yl. Interestingly, any of the vitamins were not required for growth in Ca and Ss. In all strains tested, elimination of zinc showed slow growth, suggesting that zinc ion has an important role for yeast growth. We also prepared uracil drop-out AYD (AYD-U) plates for single colony isolation and transformation selection. Only one day incubation was required for colony formation, suggesting that colony isolation and selection can be performed on AYD drop-out medium within one day. AYD drop-out media can be used for growth and screening experiments in various yeasts.

Keywords: *Medium, Nutrient, Saccharomyces, Kluyveromyces, Candida, Scheffersomyces, Yarrowia*

[PS15-5] The Impact of Saccharomyces cerevisiae M2 Virus on Host Gene Expression

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Almost all described species of Saccharomyces yeast host double-stranded RNA (dsRNA) viruses of the family Totiviridae. The S. cerevisiae L-A dsRNA virus family along with several satellite dsRNAs (M1, M2, M28 or Mlus) are widely distributed in nature. L-A helper virus encodes the capsid protein Gag, necessary for viral particle structure and Gag-Pol fusion protein, required for maintenance and replication of both dsRNAs. In contrast to L-A, M dsRNAs encode only the unprocessed precursor of the mature secreted killer toxin, which provides fitness advantages to their hosts by eliminating toxin-sensitive competitors. Simple eukaryotic organism S. cerevisiae, hosting dsRNA virus-originated killer systems, is used as attractive model for understanding the molecular mechanisms of viral infection and pathways viruses exploit to overtake the host cellular machinery.

We performed gene expression profiling on the killer yeast strain M437 (wt, HM/HM [kil K2]), harbouring M2 dsRNA, and isogenic strain M437-M2- (wt, HM/HM [kil 0]), cured from the killer virus. The presence and absence of dsRNA virus in particular yeast strains were confirmed by functional tests and verification of corresponding dsRNA by RT-PCR. The total RNA from the particular yeast cells was isolated, mRNA libraries constructed and subjected to barcoded Illumina HiSeq 2000 transcriptome high-throughput paired-end sequencing. By performing profiling of the whole transcriptomes, we examined the impact of M2 dsRNA virus on pattern of yeast gene expression. We detected host genes and pathways involved in M2 dsRNA virus maintenance and propagation as well as those important for virus-host interaction. This research was funded by a grant (No. SIT-7/2015) from the Research Council of Lithuania.

Keywords: *Saccharomyces cerevisiae, double-stranded RNA viruses, gene expression profiling, RNA-Seq*

[PS15-6] Lipid Droplets and Vacuoles of Candida albicans and Saccharomyces cerevisiae: Raman Microspectroscopy Study

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Human opportunistic pathogen Candida albicans can survive under different types of stress conditions imposed by host. After internalization by host phagocytes, the yeast cells are subjected to carbon or nitrogen deprivation. C. albicans has therefore developed high adaptability and versatility of metabolism. Storage compartments such as vacuoles and lipid droplets play a key role in adaptation to nutritional stress and have an impact on virulence. In addition, fungal vacuoles are crucial for killing host macrophages.

We used confocal Raman microspectroscopy to determine and compare chemical composition of vacuoles and lipid droplets in living cells of C. albicans and Saccharomyces cerevisiae, that have been cultivated either in nutritionally rich medium (YPD) or under the

conditions of nitrogen starvation (YNB, supplemented with proline as the sole source of nitrogen).

The Raman maps of lipid droplets indicate that nitrogen starvation caused decrease of content of fecosterol or episterol, which are intermediates of the ergosterol biosynthesis pathway, in both C. albicans and S. cerevisiae. Raman spectra also suggest the upregulated synthesis of unsaturated and saturated fatty acids or triacylglycerols in S. cerevisiae upon nitrogen starvation. The changes in composition of lipid droplets in C. albicans upon nitrogen starvation were less dramatic.

Assignment of Raman bands in the vacuoles was more difficult. However, polyphosphate that serves as a vacuolar marker, can be identified unambiguously. While in S. cerevisiae the polyphosphate content does not significantly change due to nitrogen starvation, vacuoles of the starved C. albicans cells contain approximately twofold amount of polyphosphate.

These results show that the two yeast species display different strategies when facing nitrogen starvation.

Acknowledgments

This project was supported by NPU project LO 1302 from Ministry of Education, Czech Republic.

Keywords: *Candida albicans, nitrogen starvation, Raman microspectroscopy*

[PS15-7] Putting high content screens into focus – a novel approach for performing genetic screening at electron microscopy resolution

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In recent years, genetic screens performed using high throughput fluorescent microscopes have generated large data-sets that contributed many insights into cell biology. However, such approaches cannot tackle question of ultra-structure that are below the resolution limit of fluorescent microscopy. Electron microscopy (EM) overcomes this resolution limit and generates high-resolution, ultra-structure, imaging. However, this advantage comes at a cost, as EM requires long and expensive sample preparation limiting throughput. To overcome this obstacle, we suggest a robust method to perform high(er) content screening using correlative light and EM. Our approach is based on pooling together different yeast populations for EM sample preparation and subsequent identification if each cell's genotype using fluorescent barcodes. Coupled with easy to use software for correlation, segmentation and computer image analysis, our method currently allows us to extract 15 different yeast populations from a single sample preparation. Such a methodology is not restricted to yeast and can be utilized in multiple ways to enable EM to become a powerful screening methodology.

Keywords: *microscopy, electron microscopy, genetic screening, HTEM*

[PS15-8] Profiling of host gene expression under the action of Saccharomyces cerevisiae L-A-lus and M-2 viruses

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Competitive and naturally occurring yeast killer phenotype is governed by coinfection of dsRNA viruses. The long-term relationship between the host cell and viruses appear to be beneficial and co-adaptive; however, the impact of viral dsRNA on the host gene expression has been barely investigated. In this study, we provide an overview of yeast (Saccharomyces cerevisiae) gene expression changes in M437 killer strain cured of both M-2 and L-A-lus dsRNA viruses. Transcriptome profiling by high throughput RNA-Seq provides the resolution necessary for the genome-wide determination of functional connections among genes regulated by dsRNA viruses.

We demonstrated that elimination of M-2 and L-A-lus dsRNAs affected transcriptional changes of 715 genes. In M437 [L-M-] cells 291 and 424 genes were up- and downregulated, respectively. Enriched GO terms of positively regulated genes are related to stress response, namely cellular response to oxidative stress, oxidation-reduction process and carbohydrate metabolic process. Negatively regulated genes in M437 [L-M-] cells are related to cellular amino acid biosynthetic process, cellular lipid biosynthetic process and cellular respiration. Insights on alteration of host gene expression will help to understand the biology of dsRNA mycoviruses and their impact on the host cells. This research was funded by a grant (No. SIT-7/2015) and travel grant (No. S-KEL-17/2017) from the Research Council of Lithuania.

Keywords: *Saccharomyces cerevisiae, dsRNA viruses, host gene expression, RNA-Seq*

[PS15-9] An Optimized FAIRE Procedure For Low Cell Numbers In Yeast

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Whilst FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) sequence (seq) has largely been supplanted by ATAC-seq, which offers an improved signal: noise ratio, FAIRE retains one key advantage: ATAC-seq in yeast requires the preparation of spheroplasts to permeabilise cells to the Tn5 transposase. The time and temperatures required to incubate cells during spheroplast preparation may allow epigenetic changes to occur, which is not a concern with FAIRE-seq.

We report an optimised low-input FAIRE-seq procedure to assay chromatin accessibility from yeast colonies grown on solid medium. Sensitivity, specificity and reproducibility of

the scaled-down method are comparable to that of regular, higher input amounts, assayed over a 100-fold range. The method enables epigenetic analysis of chromatin structure in small number of yeast cells and thus opens the possibility to study wild yeasts that form colonies, or those that can be isolated in sufficient amounts from environmental samples. It is the first protocol to demonstrate FAIRE in cells directly taken from colonies cultured on agar plates.

Keywords: *yeast colony, New protocol, FAIRE-seq, Low-scale*

[PS15-10] Generation of Pooled Genome-Wide Yeast Clone Libraries by CASTING

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Yeast strain libraries are highly invaluable resources for functional genomic investigations. Such resources are composed of arrayed strain collections, in which each strain has a specific modification at a different genomic locus. Existing libraries have revolutionised the study of gene expression, protein function, localisation, and protein-protein interactions. The creation of new genome-wide libraries, however, is prohibitively expensive, labour-intensive, and requires specialised lab-equipment to handle the strain arrays.

Here, we introduce a conceptually reversed approach by using a pooled format for library construction, high-throughput functional profiling, and next-generation sequencing. We present a new strategy to create genome-wide yeast clone libraries in a single reaction tube, which makes use of low-cost pooled oligonucleotide synthesis platforms and exploits an RNA-guided endonuclease to facilitate the precise, site-directed integration of a tagging cassette by homologous recombination. Functional analysis of such pooled yeast clone libraries involves their sorting (e. g. using FACS or growth competition) followed by a target-enriching next-generation sequencing protocol to characterise the identity of strains in the individual fractions.

Exemplarily we show potential applications of this format for comparative genomics, expression landscape mapping, and forward genomic screening.

Keywords: *Functional Genomics, Yeast Clone Library, Molecular Cloning*

[PS15-11] High-throughput Protein Tagging With the C-SWAT Library

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Yeast systems biology profits from several genome-wide libraries of arrayed strains, where all genes bear the same genetic modification. Existing libraries have produced invaluable knowledge on the function, fitness, and evolution of the eukaryotic cell. New biological questions will, however, demand the construction of new libraries, which is a time- and cost-intensive process. To address this limitation, we recently developed the SWAp-Tag (SWAT) methodology, which allows for the rapid and straightforward construction of new libraries by exchanging tags in a so-called acceptor or master library(Yofe et al, 2016).

Here, we present the C-SWAT master library where nearly all yeast genes have been tagged at their 3' end with an acceptor module. We also introduce a novel targeted sequencing approach called proximitySeq, to validate the genomic modification of thousands of strains in parallel without the need for colony-PCR. New libraries can be efficiently created by replacing the acceptor module with any desired sequence, including protein tags (standard and seamless (Khmelinskii et al, 2011)), mRNA tags and gene regulatory elements, in ~3 weeks.

To demonstrate the versatility of the C-SWAT library, we derived two daughter libraries with improved red and green fluorescent proteins tags. These new libraries enabled us to measure the expression of many genes previously undetectable using the original GFP library. The C-SWAT library expands the yeast functional genomics toolkit and will serve as a valuable resource to the research community.

Khmelinskii A, Meurer M, Duishoev N, Delhomme N & Knop M (2011) Seamless gene tagging by endonuclease-driven homologous recombination. PLoS ONE 6: e23794.

Yofe I, Weill U, Meurer M, Chuartzman S, Zalckvar E, Goldman O, Ben-Dor S, Schütze C, Wiedemann N, Knop M, Khmelinskii A & Schuldiner M (2016) One library to make them all: streamlining the creation of yeast libraries via a SWAp-Tag strategy. Nat Methods 13: 371–378.

Keywords: *Genome-wide yeast libraries, SWAp-Tag methodology, Novel targeted sequencing*

[PS15-12] Enhancing post-SCRaMbLE fitness: Fast-tracking synthetic yeast for industrial applications

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The construction and study of synthetic genomes are crucial for understanding the complexities of genome structure, organisation and plasticity. The Synthetic Yeast 2.0 project introduced a novel concept of forced inducible evolution and massive genomic rearrangement. This is facilitated by the incorporation of loxPsym recombination sites downstream of all nonessential genes and the subsequent recombination at these sites by an inducible Crerecombinase; induction of this LoxP/Cre recombination is termed SCRaMbLE. This system has been used to generate libraries of yeast strains with diverse genome rearrangements. SCRaMbLEed populations are generally enriched for gene duplications and inversions. Longer recombination induction periods result in an increased

frequency of recombination events, increases the postinduction population diversity, but also severely impair cell fitness due to the loss of essential genes or gene combinations. While these genomic rearrangements will facilitate a greater understanding of yeast genetics, it is currently only available in specific designer laboratory strains and are unlikely to convey industrially relevant phenotypes at a high frequency. Several studies have previously shown that subtle changes in native gene copy number can result in significant industrially relevant phenotype improvements. It has also been reported that increased ploidy may stabilise toxic euploidy effects that are generated during SCRaMbLE. In this study we set out to construct synthetic yeast 2.0 strains with increasing ploidy states (n, 2n, 3n, 4n). We aim to determine the effect of ploidy on the relative population fitness after extended periods of SCRaMbLE and to improve the concomitant enhancement in foreign DNA incorporation through loxPsymassociated recombination. In addition, we will investigate various gene copy number effects (including that of essential genes and epistatic-like effects) on industrially relevant phenotypes. In this initial part of the study, demonstrated an increased post-SCRaMbLE population viability with an increase in strain ploidy. The higher ploidy states and concomitant increased loxPsym recombination sites allowed significantly higher URA3 markercassette integration. These preliminary findings suggest that the synthetic lab strains could serve as stable, tunable gene dosage ‘addons’ to established industrial strains for further optimisation and serve as conduits for enhanced heterologous gene integration.

Keywords: *Yeast 2.0, SCRaMbLE, loxPsym*

[PS15-13] Improved Reverse Yeast 2-Hybrid Assay identifies a novel putative Mdm2-binding site in p53

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Protein-Protein interactions (PPI) are fundamental to growth and survival of cells and serve as excellent targets to develop inhibitors of biological processes such as host-pathogen-interaction and cancer cell proliferation. While several /in vitro/-based assays to screen for PPI inhibitors are available, very few use an /in vivo /assay for screening. We report an improved and validated Reverse Yeast 2-hybrid (RY2H) assay that can be used in a high throughput manner to screen for PPI inhibitors. Using the p53-Mdm2 interaction to optimize the assay, we show that the anti-cancer drug nutlin-3 is a substrate for the yeast ABC transporter Pdr5. We generated a yeast strain ABC9Δ that lacks 9 ABC transporter related genes and is highly permeable to small molecules. In the ABC9Δ strain, the p53-Mdm2 interaction inhibitors like AMG232 and MI-773 work at nanomolar concentrations in the RY2H assay. In addition, we have identified a conserved segment in the core DNA-binding domain of p53 that facilitates stable interaction with Mdm2 in yeast cells and /in

vitro/. We propose that the improved RY2H assay can also be utilized for identifying domains that stabilize PPI.

Keywords: *Reverse Yeast 2-Hybrid, Protein-Protein interactions (PPI), p53-Mdm2 interaction inhibitors*

[PS16] Systems biology of yeast and bioinformatics

[PS16-1] Comparative Analysis of Protein Abundance Studies to Quantify the Saccharomyces cerevisiae Proteome

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Global gene expression and proteomic tools have allowed large-scale analyses of the transcriptome and proteome in eukaryotic cells. These tools have enabled studies of protein abundance changes that occur in cells under stress conditions, providing insight into the regulatory programs required for cellular adaptation. While the proteome of yeast has been subjected to the most comprehensive analysis of any eukaryote, each of the existing datasets are separate and reported in different units. A comparison of all available datasets is key towards a complete understanding of the yeast proteome. We evaluated 19 quantitative proteomic analyses performed under normal and stress conditions and normalized and converted all measurements of protein abundance into absolute molecules per cell. Our analysis yields an accurate estimate of the cellular abundance of 92% of the proteins in the yeast proteome. We evaluate the variance and sensitivity associated with different measurement methods and explore the correlation of protein abundance with RNA sequencing and ribosomal profiling data. We find that C-terminal tagging of proteins has little effect on protein abundance. Finally, our normalization of diverse datasets facilitates comparisons of protein abundance remodeling of the proteome during cellular stresses.

Keywords: *Protein Abundance, Proteome, High-Throughput, Green Fluorescent Protein, Mass Spectrometry, Tandem Affinity Tag*

[PS16-2] Phenotypic Profiling in Yeast Using High-content Screening and Automated Image Analysis

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We have developed experimental and computational pipelines which combine array-based yeast genetics and automated microscopy for systematic and quantitative cell biological screens or phenomics. In one project, we use the Synthetic Genetic Array (SGA) method to introduce fluorescent markers of key cellular compartments or cell cycle progression, along with sensitizing mutations, into yeast mutant collections. We then perform live cell imaging on the mutant arrays using HTP confocal microscopy to quantitatively assess the abundance and localization of our fluorescent reporters, providing cell biological readouts of specific pathways and cellular structures in response to thousands of genetic perturbations. For automated image analysis, we developed a hybrid computational

pipeline that combines outlier detection and classical SVM-driven phenotype labeling, as well as a neural network-based approach. Our neural network, DeepLoc, was able to classify highly divergent image sets, highlighting deep learning as an important tool for expedited analysis of high-content microscopy data.

Keywords: *functional genomics, image analysis, cell biology*

[PS16-3] Integration of Structure- and Network-based Approaches for Network Pharmacology Prediction

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Drugs or drug candidates may interact with numerous molecules in the human body. Unexpected binding between compounds and proteins (i.e. off-target interactions) may result in unfavourable effects, leading therapeutic risks, negatively impacting drug development. The application of network pharmacology to predict molecular binding potential, drug reactions and toxicity caused by multiple targeting interactions is therefore expected as a promising method for evaluating polypharmacological effects. Here, we present systemsDock, a web server which integrates the results from molecular simulation and network-based analysis to assess binding potentials of a given small molecule (test compound) against proteins involved in a complex molecular signalling pathway. We developed a predictive re-scoring function to assess molecular binding poses generated by docking tools and to rank them accordingly. In a validation of classifying test compound activity by using Receiver Operating Characteristic (ROC), we obtained the values of 75% sensitivity (i.e. compounds correctly identified as active) and 76% specificity (compounds correctly identified as inactive), showing a good performance on prediction of molecular binding potential.

We used the developed systemsDock system to screen numerous test compounds over Influenza A Virus Life Cycle pathway map (FluMap) for discovering anti-influenza agents. Together with the application of our re-scoring function, the proposed screening approach is able to comprehensively characterize the underlying mechanism of a drug candidate with good accuracy, improving the prediction of drug efficacy and safety. systemsDock is freely accessible at http://systemsdock.unit.oist.jp/.

Keywords: *Computer-aided drug design., Network pharmacology., Molecular simulation.*

[PS16-4] Leucine biosynthesis is involved in regulating high lipid accumulation in Yarrowia lipolytica

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The yeast Yarrowia lipolytica is a potent accumulator of lipids and lipogenesis in this organism can be influenced by a variety of factors, such as genetics and environmental conditions. We have previously identified the involvement of the regulation of amino-acid metabolism to redirect flux to lipid accumulation [1]. We have followed up on this using a multifactorial study, thereby elucidating the effects of both genetic and environmental factors on regulation of lipogenesis in Y. lipolytica and identifying how two opposite regulatory states both result in lipid accumulation [2].

The study involved the comparison of a strain overexpressing diacylglycerol acyltransferase (DGA1) with a control strain grown under either nitrogen or carbon limited conditions. A strong correlation was observed between the responses on the levels of transcript and protein. Combination of DGA1 overexpression with nitrogen limitation resulted in high-level of lipid accumulation accompanied by downregulation of various amino acid biosynthetic pathways, with leucine in particular, and these changes were further correlated with a decrease in metabolic fluxes.

The downregulation in leucine biosynthetic pathway genes was supported by a measured decrease in the level of 2-isopropylmalate, an intermediate of leucine biosynthesis. Combining the multi-omics data with putative transcription factor binding motifs uncovered a contradictory role for TORC1 in controlling lipid accumulation, likely mediated through 2-isopropylmalate and a Leu3-like transcription factor.

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Keywords: *Yarrowia lipolytica, regulation, metabolism, biotechnology, genome-scale modelling, integrative data analysis*

[PS16-5] Systems Level Study of S. cerevisiae Mutant Cells Demonstrating Different RNA Polymerase III Activity

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S. cerevisiae, efficiently fermenting sugar and producing ethanol, serves as a model for studying cancer. Both yeast and tumor cells rely on increased glucose uptake and high

glycolytic activity. In addition, rapidly proliferating cancer cells not only accelerate glycolysis but also show abnormally elevated abundance of RNA polymerases I (RNAP I) and III (RNAP III) transcripts.

Regulation of RNAP III, which is responsible for tRNA synthesis in yeast S. cerevisiae in response to carbon source is mediated by general RNAP III- repressor, Maf1. Maf1 driven repression occurs on non-fermentable carbon source. The maf1 Δ strain has perturbations in gluconeogenesis [1]. The phenotypic effect of Maf1 deletion is suppressed by G1007A point mutation in the second largest RNAP III subunit C128 [1]

The two mutated strains have different preferences towards carbon sources.

The maf1 Δ, in which transcription of the gluconeogenic genes FBP1 and PCK1 is down regulated on glycerol, has reduced HXT6/7 transcript levels [3]. rpc128-1007 mutant, which grows poorly in the presence of a high concentration of glucose but has unperturbed glucose sensing via the major glucose signalling pathway Snf3/Rgt2, shows increased transcripts of HXT6/7 and HXT2 transporters, (which are considered to respond only to low glucose concentrations), regardless of the growth conditions either in the presence of fermentable or non-fermentable carbon source [2]

To survey proteome changes in relation to glucose utilization, we performed a comparative label-free quantitative study in response to high glucose concentration in maf1 Δ and rpc128-1007 strain. The changes in proteome demonstrate that the economy of protein production and glucose metabolism is central to cell physiology. Although glycolysis is accelerated in maf1 Δ it does not lead to an enhancement of ethanol production, channelling carbon to other metabolic end products.

The presented study provides an elegant example of using systems level approach in combination with targeted metabolic and molecular biology analysis, such as RT-PCR quantification of mRNA steady-state levels, for successful deciphering of metabolism in Saccharomyces that can further serve as a template for design of strains for increased production of industry-relevant commodities in yeast cells designed “a la carte”. This study was supported by National Science Centre, Poland grant no. 2012/05/E/NZ2/00583

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Keywords: *RNAP III activity, tRNA, glucose signaling, RNAP II gene regulation, metabolism, proteomics*

[PS16-6] More Than One Way In - Three Gln3 Sequences Required To Relieve Negative Ure2 Regulation and Support Nuclear Gln3 Import

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Gln3 is responsible for Nitrogen Catabolite Repression-sensitive transcriptional activation in yeast. In nitrogen-replete medium, Gln3 is cytoplasmic and NCR-sensitive transcription

repressed. In nitrogen-limiting medium, in cells treated with TorC1 inhibitor, rapamycin or glutamine synthetase inhibitor, methionine sulfoximine (Msx), Gln3 becomes highly nuclear and NCR-sensitive transcription derepressed. Previously, nuclear Gln3 localization was concluded to be mediated by a single nuclear localization sequence, NLS1. Here we show that nuclear Gln3-Myc localization is significantly more complex than previously appreciated. We identify three Gln3 sequences, other than NLS1 that are highly required for nuclear Gln3-Myc localization. Two of the sequences exhibit characteristics of mono- and bi-partite NLS sequences. Mutations altering these sequences are partially epistatic to a ure2 deletion. The third sequence, the Ure2 relief sequence, contains no NLS homology. Phosphomimetic aspartate substitutions for serine residues in the Ure2 relief sequence pleiotropically abolish nuclear Gln3-Myc localization in response to both limiting nitrogen and rapamycin treatment. In contrast, these Gln3 responses are normal in parallel serine to alanine substitution mutants. A ure2 deletion is epistatic to the aspartate to serine substitutions in the Ure2 relief sequence. These observations demonstrate that Gln3 responses to specific nitrogen environments potentially occur in multiple steps that can be genetically separated. At least one general step, that which is associated with the Ure2 relief sequence, may be prerequisite for responses to the specific stimuli of growth in poor nitrogen sources and rapamycin inhibition of TorC1. Supported by NIH grant GM-35642-27.

Keywords: *nitrogen catabolite repression, TorC1, Gln3, Ure2, rapamycin, methionine sulfoximine, nuclear import, protein phosphorylation*

[PS16-7] Memory of stress response in S. cerevisiae

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When a cell undergoes an external stress, it responds by activating specific genes with a certain dynamics. In the budding yeast for instance, better tolerance or faster transcriptional response have been described for successive oxidative or nutritive stresses, respectively. Various hypotheses have been proposed to explain the mechanisms behind those different effects such as the influence of the chromatin or the involvement of specific cytoplasmic factors. But it still remains unclear how such memories are established.

To address those questions, we have investigated the response to hyperosmotic stresses in S. cerevisiae. We have used a microfluidic system to subjugate the cells to short repeated stresses while making single-cell measurement of the STL1 gene, tagged with fluorescence. This gene is located in the subtelomere of the chromosome IV, a domain of low transcriptional activity in non-stress conditions. We defined “mothers” the cells that receive the first stress and “daughters” the cells born from such stressed mothers. Our results show that the mothers adapt to repeated stresses and we have named this phenomenon “memory effect”. This memory effect is transmitted to the daughters, even though they have never experienced a stress before.

We have moved the gene of interest to a domain of higher transcriptional activity, which has interestingly led to a decrease in the gene’s activity upon stress and the loss of the memory effect. This suggests an involvement of the chromatin environment on the

response to repeated stresses. Moreover, we have found a dynamical variability among cells which may or may not respond to several consecutive stresses. As shown by stress response modelling, this phenomenon could be explained by a variable delay among the yeast between the sensing of the stress and the start of the genetic response.

We found that even in response to short hyperosmotic stresses, the same population of yeast develop a memory of the stress, which is transmitted to the first generation of progeny. Our study show that this memory is gene-position dependent. This suggests an evolutionary organization of the stress response genes in the nucleus that might be involved in the emergence of phenomenon such as the memory effect. This work could serve as a basis to explain similar phenomena in other organisms.

Keywords: *memory, evolution, single-cell, microfluidics, variability, microscopy, nuclear organization*

[PS16-8] A Novel Platform for Secretory Production of Recombinant Proteins in Saccharomyces cerevisiae

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In spite of the higher preference of Saccharomyces cerevisiae as an expression host for the secretory production of recombinant proteins, only a limited number of secretion signals are available. To expand the repertoire of effective secretion signals in S. cerevisiae, we developed a target protein-specific translational fusion partner (TFP) screening system that involves recruitment of an optimal secretion signal and fusion partner from genome. Around 4 □ 103 TFP library was constructed from a genomic and truncated cDNA library by using the invertase-based signal sequence trap technique. The efficiency of the system was demonstrated using two rarely secreted proteins, human interleukin (hIL)-2 and hIL-32. Optimal TFPs for secretion of hIL-2 and hIL-32 were easily selected, yielding secretion of these proteins up to hundreds of mg/L. Moreover, numerous uncovered yeast secretion signals and fusion partners were identified, leading to efficient secretion of various recombinant proteins. Selected TFPs were found to be useful for the hypersecretion of other recombinant proteins at yields of up to several g/L. This screening technique could provide new methods for the production of various types of difficult-to-express proteins.

Keywords: *Translational fusion partner, Saccharomyces cerevisiae, Protein secretion*

[PS16-9] Computational Analysis of the Glycolytic Gene Expression Patterns in Different Saccharomyces cerevisiae Strains Grown in Solid State Fermentation Conditions

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Glycolytic enzyme genes are the highly expressed and subjected to transcriptional regulation by various regulators. Expression profiles of glycolytic genes may vary depending on the growth conditions and yeast strains. In this study, the expression profiles of glycolytic genes in three different Saccharomyces cerevisiae strains that are grown under solid state fermentation (SSF medium) conditions, were analyzed using bioinformatics tools. The row data on the transcriptome analysis were downloaded from EMBL-EBI (study ID: PRJNA212389). Transcript levels of each glycolytic genes were analyzed using Trimmomatic, Tophat2 and Featurecounts programs. Analysis of RNA-seq data indicates that certain glycolytic genes were subjected to rapid reprogramming for transcription in response to SSF. It appears that transcription of HXK1, GLK1 and genes that encodes the key factors for the trehalose (TPS1, TPS2 and NTH1) and glycogen metabolism (PGM2, UGP1, GSY1, GSY2 and GPH1) is down regulated rapidly when the yeast strains inoculated into SSF medium. At the same time, genes that involves in glycerol metabolism (GPD1, GPP1/RHR2 and GPP2/HOR2) is activated. At the second stage (60 min after inoculation), while the transcription of GPD1 and GPP2/HOR2 decreases, transcription of TPI is activated. Transcription of genes involves in trehalose and glycogen metabolism remains at basal level during this stage. At the third stage (180 min after inoculation), transcription of HXK1, GLK1 and genes involves in trehalose and glycogen metabolism elevate to their initial level. Moreover, transcription of TDH1, PDC1, ADH1 and FBA1 is highly activated after second and third stage of SSF. Activation of genes that has a significant function in the lower glycolytic pathways suggest that yeasts grown under SSF conditions activates NADH biosynthesis for redox balance too. TDH1 encodes a multi-functional peptide that also present in the yeast cell wall and also secreted to growth medium. The transcription of GCR1, a major activator of glycolytic genes, activated by 2-fold in SSF medium and remains at activated level. In addition, we identified a remarkable changes in the expression of glucose transporter genes too. In conclusion, it appears that growth in SSF medium results with rapid changes in the transcription of glycolytic genes in S. cerevisiae. It seems that NADH production via glycerol and ethanol synthesis are the essential processes for growth in SSF medium for S. cerevisiae.

Keywords: *Glycolytic Genes, RNA-seq, Saccharomyces cerevisiae*

[PS16-10] Computational Investigation of TOR1 Gene Structure and Expression Profiles in Different Yeast Species

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TOR1 gene encodes serine threonine kinases which regulate cellular processes such as cell growth, translation, transcription, lipid and protein synthesis. TOR pathway is evolutionary conserved in all eukaryotes. TOR1 functions in 2 distinct protein complexes, called TORC1 and TORC2. Its major upstream regulators are growth factors, stress, nutrient, cellular energy levels and hypoxia. In this research, we have investigated the structure and functional relatedness of TOR gene and its expression profiles in different yeasts using bioinformatics tools. We have used available genome databases and RNAseq data of S.

cerevisiae, S. bayanus, S. mikatae, S. paradoxus, C. albicans and S. kluyveri. It is found that TOR1 sequence has divergence among the yeast species analyzed in this study. While C. albicans has 65% similarity to S. cerevisiae TOR1 sequence, S. paradoxus and S. bayanus show 99% and 82% similarities, respectively. We found that TOR1 expression level is the highest in S. bayanus when compared to other yeasts according to RNAseq analysis results. To find out its reason, TOR1 promotor regions and transcription factors associated with it were investigated. The promoter region sequence of TOR1 in S. bayanus shows moderate level differences comparing S. cerevisiae. Interestingly, we have found that transcription factors that associate with the TOR1 promoter region also overexpressed in S. bayanus. Moreover, RNAseq analysis results clearly indicate that S. bayanus relatively overexpressed certain stress response genes comparing S. cerevisiae. The results of this research revealed that adaptation to different environmental and stress conditions might be linked to TOR signaling pathway.

Keywords: *TOR1, S. bayanus, RNA-seq, Stress response*

[PS16-11] Using Genome Scale Metabolic Model to Explore the Lipid Producing Machinery of Rhodosporidium toruloides

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The red basidiomycetous yeast Rhodosporidium toruloides can accumulate lipids to more than 50% of their cell dry weight when cultured on xylose. Lipid metabolism in R. toruloides is distinguished by the presence of ATP:citrate lyase, a cytoplasmic malic enzyme, phosphoketolase, enzymes of mitochondrial beta-oxidation and fatty acid synthase with structure different from other fungal species.

Here we report the generation of the first Genome Scale Metabolic Model (GEM) of R. toruloides, which will be used to further understand the lipid metabolism in this yeast as well as integration of proteomics data and identification of relevant targets for genetic engineering. The RAVEN toolbox was used to create a first draft of R. toruloides model based on protein homology to Saccharomyces cerevisiae model yeast 7.6 and to Yarrowia lipolitica model iYali4.01. The gap filling tool Meneco was used for model completion and final manual curation of the model was performed in RAVEN. The final model draft contains around 2500 metabolites, 3000 reactions and 826 genes.

The model was used to describe metabolic fluxes in R. toruloides during lipid production from both glucose and xylose. Substrate consumption and growth rates predicted by the model were validated by experimental data. Comparative flux analysis of R. toruloides grown on glucose and xylose were then integrated with proteome data. Finally, the model was used to identify targets for metabolic engineering in order to improve xylose conversion into lipids in R. toruloides.

Keywords: *lipid metabolism, genome scale metabolic model, xylose*

[PS17] Yeast comparative and evolutionary biology

[PS17-1] Mechanism and Impact of De Novo Gene Emergence Across 15 Yeast Genomes

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How new genes and new protein functions arise is a fundamental question in evolution. The creation of novel functions using existing genes as raw material (gene duplication, gene fusion etc.), once considered the only plausible path to innovation, has been extensively studied. Nonetheless, the discovery of de novo gene emergence from previously noncoding sequences proved that novel genes can also evolve "from scratch". A decade after the first de novo genes were found in Drosophila, the underlying molecular mechanisms as well as the overall impact of de novo emergence on genome evolution are still a matter of debate.

We developed a comprehensive methodology combining extensive homology searches, protein evolution simulations and machine learning to, for the first time, reliably identify de novo protein-coding genes across multiple genomes. We applied our method on 15 genomes from the 2 densely sampled yeast genera, Lachancea and Saccharomyces sensu stricto, and complemented the in silico analyses with Mass Spectrometry proteomics experiments. We found that the rate of gene emergence is steady within each genus but varies 10-fold between the 2 genera. Our results suggest that de novo genes have originated from noncoding regions that are significantly more GC-rich compared to the intergenic genome average. De novo genes were predominantly found divergently oriented relative to their neighbours, suggesting that their emergence was likely driven by divergent transcription from bidirectional promoters. In Saccharomyces, where data were available, de novo genes were found associated to recombination hotspots. These findings lead us to propose that in yeasts, the combination of 1) high GC-content sequences around bidirectional promoters, a result of GC-biased gene conversion following Double Strand Breaks, and 2) constant divergent transcription of these same regions, provide conditions that favor de novo gene emergence by generating transcripts with lower probability for AT-rich stop codons and thus higher probability for ORF formation. High GC-content in these transcripts could also lead to higher translational efficiency which would further increase their protein-coding potential.

Keywords: *de novo gene emergence, novel genes, evolutionary innovation, yeast genome dynamics, Taxonomically Restricted Genes, Orphan genes*

[PS17-2] Towards a Species-wide View of the Genetic Architecture of Traits

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One of the main goals in modern genetic is to understand the relationship existing between genotype and phenotype. However, it is still difficult to precisely address molecular bases underlying complex traits. Are all the inherited traits mainly governed by additivity? What is the degree of non-additivity and how do they affect trait heritability in a population? A better understanding of the genetic architecture of complex traits requires both a precise estimation of genetic components governing phenotype and the completion of a study on a broad panel of individuals and traits, representing the totality of the species diversity.

To obtain this unbiased view of traits genetic architecture, the yeast Saccharomyces cerevisiae represents a powerful and unique model with its ease of use and the number of tools available. With the recent resequencing of more than 1,000 complete genomes of natural isolates in our lab, this dataset gives us the most complete view of the phenotypic and genetic diversity of a eukaryotic model.

Digging into yeast genetic architecture of trait has several targets. First, we performed a breakdown of the genetic components responsible for the phenotypic variation. In a second time, a precise estimation of the expressivity of traits on different genetic backgrounds has been be carried out. Finally this work allowed to characterize both rare and common variants associated with specific traits.

We are currently focusing on the genetic architecture of traits in hybrid cells. To do so, we generated a diallel cross panel of 3,025 hybrids resulting from the systematic intercrossing of 55 haploid strains available in both mating type. Phenotyping of these hybrids has been carried out on 53 stress related traits (20 compounds with different concentrations) giving 160 325 cross/trait combination.

To estimate the proportion of additivity versus non-additivity in phenotypes and to obtain a view on the importance of dominance, we looked at heterosis depicting the phenotypic shift of an hybrid compared to the mean of its two parents. Diallel panel offers a good opportunity to evaluate the genetic variance components in hybrids using combining abilities which also grants an estimation of broad and narrow-sense heritability.

The last step on the diallel hybrid panel analysis has been to perform genome-wide association studies (GWAS) to uncover common additive variants responsible for particular phenotypes.

Keywords: *Genetic Architecture of Traits, Non-additivity, Heterosis, Diallel Cross, Combining abilities, Genome-wide Association Study*

[PS17-3] Evolutionary Restoration of Fertility in an Interspecies Hybrid Yeast, by Whole-Genome Duplication After a Failed Mating-Type Switch

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Many interspecies hybrids have been discovered in yeasts, but most of these hybrids are asexual and can only replicate mitotically. Whole-genome duplication has been proposed as a mechanism by which interspecies hybrids can regain fertility, restoring their ability to perform meiosis and sporulate. Here, we show that this process occurred naturally during the evolution of Zygosaccharomyces parabailii, an interspecies hybrid that was formed by mating between two parents that differed by 7% in genome sequence and by many interchromosomal rearrangements. Surprisingly, Z. parabailii has a full sexual cycle and is genetically haploid. It goes through mating-type switching and auto-diploidization, followed by immediate sporulation. We identified the key evolutionary event that enabled Z. parabailii to regain fertility, which was breakage of one of the two homeologous copies of the mating-type (MAT) locus in the hybrid, resulting in a chromosomal rearrangement and irreparable damage to one MAT locus. This rearrangement was caused by HO endonuclease, which normally functions in mating-type switching. With one copy of MAT inactivated, the interspecies hybrid now behaves as a haploid. Our results provide the first demonstration that MAT locus damage is a naturally occurring evolutionary mechanism for whole-genome duplication and restoration of fertility to interspecies hybrids. The events that occurred in Z. parabailii strongly resemble those postulated to have occurred to cause ancient whole-genome duplication in an ancestor of Saccharomyces cerevisiae.

Keywords: *Hybrid yeast, Mating-type switch, Zygosaccharomyces parabailii, Fertility restoration*

[PS17-4] Understanding Adaptation and Fitness Trade-offs in Yeast

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The study of how adaptive mutations affect fitness in their evolution environment and trade-off in alternative environments is essential for understanding the evolutionary process. However, it is only recently that advances in sequencing technology have provided an unprecedented opportunity to study the genetic and physiological basis of adaptation and fitness trade-offs at the molecular level.

In our previous work, we developed a DNA barcode-lineage system in yeast to track their evolutionary dynamics under a glucose-limited serial transfer condition. This system enabled further isolation of thousands of independently evolved lineages from the same experiment and identification of their adaptation-driving mutations.

In this work, I have developed a high-throughput approach to investigate the physiological basis of adaptation and trade-offs in these evolved lineages, by decomposing their fitness into distinct physiological phases in a highly quantitative manner. To achieve this, I measured the fitness of thousands of evolved lineages in bulk under experimental conditions modified from the Evolving Condition (EC, which includes lag, fermentation and respiration phases, but not stationary phase), where the lengths of fermentation, respiration, and stationary phases were systematically varied. Qualitatively, I discovered that self-diploidized adaptive lineages (diploids) and adaptive haploids differed in their adaptive strategies: diploids only gained fitness during fermentation; however, adaptive haploids gained fitness from both fermentation and respiration. Quantitatively, while all adaptive lineages had similar fitness changes during fermentation, their fitness changes during respiration were determined by their genetic basis and were strongly correlated with their fitness under the EC. Moreover, I observed an anti-correlation between respiratory fitness change and stationary fitness change, indicating an intrinsic trade-off between respiratory growth and survivability during stationary phase. Lastly, since mutants responded to different conditions in quantitatively distinct ways, I was able to predict genotypes from their collective set of fitness measurements.

Our study demonstrates that fitness measurements under rationally-designed conditions are a powerful way to study the physiological basis underlying adaptation and trade-offs, and to cluster genetically/physiologically similar lineages without detailed molecular analysis.

Keywords: *Adaptation, Serial transfer, High-throughput fitness decomposition, Fitness trade-off, Physiology*

[PS17-5] Yeast Communities on Fruits and Berries of Lithuania

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The distribution of yeast on fruits and berries is defined by continuum of factors: geographic location of plant species, ripening stage, application of agrochemicals and many others. Yeast inhabiting the fruit surface are either parasitic, neutral or beneficial to plants as symbionts or antagonists. The interactions between different yeast species may influence the structure of microbial communities inhabiting the fruit surface and through either direct or indirect impact on plant can mediate many ecological and evolutionary processes.

To investigate the diversity of yeast occurring on different fruits and berries (apples, pears, plums, currants, grapes, chokeberries, buckthorns, rowans, etc.), cultured in distinct regions of Lithuania, we applied culture-dependent and culture-independent approaches. First of them allowed us to isolate and identify yeast species prevalent on plants and capable to survive the fermentation conditions, while second provide global picture on the structure of microbiota on plant. During this study, we identified eukaryotic microorganisms common for many plants as well as yeast inhabiting the surface of

particular fruits and berries only. The impact of biogeography and environmental factors on the spreading of yeast was assessed by analysing fruits and berries samples from distant regions of Lithuania (north, south, west and east) for two years (2015 and 2016). In addition, complex interactions between selected yeast species were observed, which may condition yeast distribution as well as structure and dynamics of population. This research was funded by a grant (No. SIT-7/2015) from the Research Council of Lithuania.

Keywords: *Plant microbiota, Yeast diversity, Biogeography*

[PS17-6] Predator Yeasts: Genomics and Molecular Biology of Necrotrophic Killer Yeasts

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Yeasts of the genus Saccharomycopsis can be isolated from diverse habitats around the globe. They exhibit a unique predacious behaviour, which allows them to feed on and kill suitable fungal prey cells. This is an act of nectrophic mycoparasitism, which is induced under starvation conditions. During predation prey cells are recognized and a penetration peg is formed, with which the prey cell is penetrated and killed. The host range is wide and includes yeasts, such as Saccharomyces cerevisiae and Candida albicans, but also filamentous fungi, e.g. Ashbya gossypii. Starvation of predator yeasts can be induced solely by the lack of methionine in the growth medium. We aim at understanding the biology of predation and characterize molecular pathways and genes required for successful killing of prey cells. To this end we have generated draft genomes of five predator yeasts: Saccharomycopsis fodiens, S. fermentans, S. crataegensis, S. schoenii and Saccharomycopsis spec. The genome sizes range from 12 Mb to 15 Mb. The genome data, which also include several contigs with telomeric repeats, suggest that loss of genes required for sulphate assimilation is causing methionine auxotrophy within Saccharomycopsis species. Interestingly, genomic signatures suggest that Saccharomycopsis species are part of the CTG clade, which reassigned the CTG codon from leucine to serine, e.g. also in C. albicans. This has guided our molecular approach towards tool development for studying predator yeasts. We have developed synthetic markers, e.g. SAK1 providing resistance against the antibiotic G418. Genome profiling indicated the presence of transposons and of gene families encoding proteins that may play a major role for predacious behaviour. This includes genes encoding proteins for cell-cell adhesion, so called flocculins; genes for cell wall degrading enzymes, e.g. chitinases; and proteases. These and other morphogenesis genes required for penetration peg formation offer excellent target genes to analyze predatory behavior in Saccharomycopsis. In order to quantify predation efficiency we have developed a quantitative predation assay. This is based on quantifying CFU of S. cerevisiae grown on a lawn of predator yeast cells.

Keywords: *fungal pathogens, Host-pathogen interaction, Comparative and evolutionary genomics*

[PS17-7] Strategy for Elimination of dsRNA Viruses from Yeast

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L-A dsRNA viruses of S. cerevisiae belongs to Totiviridae family, which represents viruses widespread among fungi and protozoa. L-A genome encodes a major capsid protein Gag and RNA-dependent RNA polymerase, which is expressed as a Gag-Pol fusion protein due to the ribosomal frameshift event. In some yeast strains, L-A coexists with its satellite virus called M. dsRNA genome of satellite virus encodes a preprotoxin, the precursor of mature secreted toxin. L-A viruses spread horizontally by cell-cell fusion and cell division. The L-A virus does not noticeably affects phenotype or growth rate of the host.

Comparative transcriptomic studies of yeast containing L-A virus and yeast lacking it could reveal molecular mechanisms and decipher cost of resources of virus maintenance. To perform these studies an effective and fully controllable method for L-A elimination from cells is needed. Three approaches were elaborated: treatment by heat, cyclohexamide, and 5-fluoruracil. All listed treatments are cytotoxic for yeast and posed to false positive hits in -omics experiments.

Our group developed novel strategy for L-A elimination from yeast. It was demonstrated that overexpression of capsid protein Gag leads to an elimination of L-A dsRNA from a cell. Furthermore, overexpression of Gag proteins from some different types of L-A viruses causes the same effect of virus elimination. The collected data brings to light evolutionary relationships between various types of L-A viruses, presumably indicating of shared molecular mechanisms involved in virus maintenance.

This work was supported by Research Council of Lithuania under the program SIT-7/2015.

Keywords: *dsRNA viruses, virus elimination, L-A virus*

[PS17-8] New Double-stranded RNA Viruses From Saccharomyces sensu stricto

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Double-stranded RNA (dsRNA) viruses belonging to Totivirus genus are widely distributed in Saccharomyces genus. This group includes species ranging from the industrially important yeast Saccharomyces cerevisiae to those widely spread in nature. As member of the Totivirus genus, L-A virus contains a linear non-segmented dsRNA genome comprising two overlapping genes: gag, which encodes major structural protein Gag, and gag-pol which encodes Gag-Pol fusion protein, formed by ribosomal frameshifting. Gag-Pol has transcriptase and replicase activities necessary for maintenance of its own genome. Some of Saccharomyces group species along with L-A dsRNA virus possess smaller dsRNAs, called M satellites. These dsRNAs usually encode secretable proteins, known as toxins, able to kill a broad spectrum of different yeast. While there are indications on relationship between certain L-A virus maintaining a distinct type of satellite dsRNA, the overall evolutionary picture remains to be resolved.

We developed universal strategy to obtain full length L-A and M dsRNA genomes form yeast. Complete viral dsRNA genomes can now be cloned, analyzed and sequenced from any yeast strain by means of enzymatic manipulations on total or fractioned RNA content. Focusing on yeast isolated from natural environment, we have identified previously undescribed L-A variants along with brand new M satellites, encoding proteins possessing killer activity. The obtained data suggest that each L-A virus variant can specifically maintain a distinct type of satellite dsRNA. Moreover, we identified clusters of L-A variants which are specific to certain Saccharomyces sensu stricto species. These findings provide an important insight on evolution of L-A viruses and respective M satellites in Saccharomyces.

This work was supported by Research Council of Lithuania under the program SIT-7/2015.

Keywords: *Killer yeasts, L-A virus, M satellite*

[PS17-9] Genome Assembly and Annotation of the Pathogenic Yeast Magnusiomyces capitatus

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The yeast Magnusiomyces capitatus belongs to a deeply branching lineage of Saccharomycotina. This species is an emerging opportunistic pathogen frequently causing fatal infections in neutropenic patients. Recently, we have demonstrated that most of the mitochondrial protein-coding genes in this yeast contain mobile insertional elements dubbed byps. These insertions remain in mature mRNAs but are ignored during proteosynthesis by programmed translational bypassing [1]. To investigate this unusual phenomenon in more detail we sequenced, assembled and annotated the 20 megabase genome sequence of the M. capitatus strain NRRL Y-17686 (CBS 197.35). The genome assembly is based on a combination of the Illumina short reads (HiSeq2000) and long reads acquired from the PacBio and MinION technologies. The genome sequence has been

assembled into 4 chromosomal contigs terminating with arrays of telomeric repeats on both sides. A preliminary annotation aided by RNA-seq data has revealed over 6000 protein-coding genes. The reference genome sequence provides a platform for further functional studies of M. capitatus and may be instrumental for identification of nuclear genome adaptations that could be associated with programmed translational bypassing in mitochondria and mobility of byp elements.

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Keywords: *genome, mitochondria, Magnusiomyces capitatus, translational bypassing*

[PS17-10] The Genetic Basis for Gamete Inviability

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Sexually reproducing organisms are dependent on the production of gametes for the continuation of their genetic lineage. Therefore, the ability to undergo a successful meiosis, producing viable and fully functional gametes is critical; failure to do so may result in weak or inviable offspring and the end of the lineage. Compounding on the difficulty to pass alleles on to the next generation, interactions between different alleles may also result in sub-optimal gametes. To investigate the underlying genetics behind why gametes are inviable we have constructed six hybrids spawning from crosses between highly diverged representatives of four Saccharomyces cerevisiae lineages. We recently published reference quality genome assemblies for the four parents and these end-to-end assemblies give us a thorough understanding of all the genetic differences in the hybrids, from single nucleotide polymorphisms to structural variation. Thanks to this, we are in a position to accurately describe how gamete viability in a hybrid is dependent on the genetic makeup of the parents. By dissecting and whole genome sequencing 2,500 gametes from each of the six hybrids, we are producing a resource of 15,000 gametes with varying viability and fitness. Using the sequence data we are exploring the impact of the recombination landscape, aneuploidies and genetic interactions on gamete inviability, and relating these phenomena to underlying genomic differences between the parents. Numbers and types of aneuploidies varied across gametes depending on parent combinations and genetic distance between parents. Aneuploidies correlate well with the gamete inviability but the majority of inviable

gametes are not explained by this. We are currently exploring the effect of the recombination landscape on gamete viability and fitness, and investigating the role of allele-allele interactions.

Keywords: *Gamete inviability, fitness, genomic variation, meiotic recombination, aneuploidies*

[PS17-11] Cooperation and division of labour in Saccharomyces cerevisiae

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Division of labour between different specialized cell types is a hallmark of biological complexity in multicellular organisms. However, it is increasingly being recognised that division of labour also plays an important role in the lives of predominantly unicellular organisms. Saccharomyces cerevisiae displays several phenotypes that could be considered a division of labour, including quiescence, apoptosis and biofilm formation, but they have not been explicitly treated as such. We discuss each of these examples, using a definition of division of labour that involves phenotypic variation between individuals within a population, cooperation between individuals performing different tasks, and maximisation of the inclusive fitness of all individuals involved. We then propose future research directions and possible experimental tests using S. cerevisiae as a model organism for understanding the very first stages of the evolution of division of labour.

Keywords: *sociomicrobiology, cell types, altruism, differentiation, cooperation*

[PS17-12] Yeast population genomics reveal origin and evolution of a classic model organism

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The baker’s yeast S. cerevisiae has had a long association with human activity, leading to the idea that its use in fermentation leads to its domestication. However, recent studies revealed that S. cerevisiae has a rather ubiquitous distribution in the wild, not limited to human-associated environments, showing that its history goes far beyond its association with humans.

In the past decade, we applied population level sequencing to thousands of S. cerevisiae strains to further illuminate the population structure and the impact of human activity. Many breeds associated to specific human process have specific genomic signatures likely driven by adaptation to industrial environments. In parallel, genome analysis of highly diverged wild lineages that predate domestication is consistent with south East Asia as the geographic origin of S. cerevisiae and support a single out-of-China origin followed by several independent domestication events. While domesticated isolates diverge by genome

content, ploidy and aneuploidy variation, wild isolate genome evolution is mainly driven by the accumulation of single nucleotide variants.

Furthermore, we use long-read sequencing to generate end-to-end genome assemblies for strains representing major subpopulations of S. cerevisiae and its wild relative S. paradoxus. These population-level high-quality genomes with comprehensive annotation enable precise definition of chromosomal boundaries between cores and subtelomeres and a high-resolution view of evolutionary genome dynamics. We observed contrasting evolutionary dynamics across the genomic landscape between S. cerevisiae and S. paradoxus. Taken together, many of these observed differences probably reflect the influence of human activities, which sheds new light on why S. cerevisiae, but not its wild relative, is one of our most biotechnologically important organisms.

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Keywords: *population genomics, domestication, structural variation*

[PS17-13] Natural Genetic Variation in Saccharomyces cerevisiae Reveals Diet-Dependent Mechanisms of Chronological Life Span Control

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Aging is one of the most fundamental processes of life. As it is a leading risk factor for many common diseases in humans, research on the molecular mechanisms involved in this process is expected to lead to a better understanding of age-associated diseases and to promote the development of health and/or life span extending strategies. Although aging-related mechanisms have widely been investigated using deletion collections isogenic the reference strain of Saccharomyces cerevisiae, natural isolates have been poorly exploited to address this research challenge. So far, the vast majority of age related studies have been performed in glucose-limited conditions. The fact that human beings are mostly living in rich-diet conditions, might limit the transferability of the findings in yeast. To investigate the impact of both genetic variability and growth condition on life span regulation, we used a collection of natural isolates of S. cerevisiae aged under various environmental conditions. Through the analysis of a segregating population, we found that genes governing the regulation of life span are highly dependent on the glucose concentration.

While we identified the RIM15 gene, encoding a protein kinase, to modulate life span in calorie-restricted conditions, we could not detect its regulatory effect in rich-diet condition, where instead a single mutation in the SER1 gene increases the longevity. By comparative studies of different sugars, we determined the carbon source to be crucial for life span modulation. We found that the use of the trisaccharide raffinose (Galactose-Glucose-Fructose) as sole carbon source increases life span to the same extend as calorie restriction. We notably identified that the presence of members of the MEL gene family is involved in this increase. These MEL genes are usually found in the subtelomeric regions of few strains only, and encode enzymes able to cut the bound between galactose and glucose in the raffinose sugar. Using a targeted metabolomics approach, we validated that strain containing a MEL gene can use galactose, glucose and fructose coming from raffinose whereas strains lacking such gene will be restricted to the use of fructose as carbon source, while the galactose-glucose disaccharide remains in the medium. Taken together, the data proves that aging studies in natural yeast variants under different environmental conditions open new avenues for deciphering the genetic and molecular basis of life span control.

Keywords: *Population genomics, Linkage mapping, Aging mechanisms*

[PS17-14] Adaptation of Yeast to Anthropogenic eEnvironments Using Comparative Genomics

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The diversity of alcoholic beverages developed by Man around the world is a remarkable outcome of history. These fermented beverages rely on the same unicellular fungi: Saccharomyces cerevisiae, which is also found in other fermented foods and natural resources. The development of these fermented beverages has shaped the diversity of this yeast species (Legras et al. 2007).

Using genomic data from 82 individuals from these various environments (biological ageing, wine fermentation, sugar cane fermentation, fermented milk, oak...), we could show that S. cerevisiae strains have gained traits reflecting the constraints imposed by natural or artificial selection in several anthropogenic ecological niches. Our results indicate that the improvement of various traits have been achieved in the different populations through different mechanism: mutation, hybridization with other yeast species or horizontal gene transfer from other yeast genera, and left specific signatures in their genomes leading to modern domesticated lineages such as Flor strains (Coi et al 2017). We present here multiple clusters of genes transferred horizontally from distant genus, genes showing evidence of positive selection and genomic regions with signature of selective sweeps

specific to the different populations. In several cases we could associate this genetic features to specific phenotypes traits such as for flor or cheese strains.

Legras J-L, et al. (2007). Molecular ecology, 16, 2091–2102.

Coi AL, et al. (2017) Molecular Ecology, 38, 42–49.

Keywords: *Saccharomyces cerevisiae, Population genomics, adaptation*

[PS17-15] Disadvantageous Pleiotropic Effect of MKT1S288c in Yeast Saccharomyces cerevisiae

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Mkt1 is a putative endonuclease that it is involved in posttranscriptional regulation of many stress related transcripts. MKT1 was found as a causal gene for several stress-related quantitative traits, including drug resistance, ethanol tolerance, sporulation efficiency and high temperature growth. These studies were conducted by comparing an inferior S288c isogenic laboratory strain and a superior non-related strain. Allele MKT1S288c has two mutations, D30G and K453R, absent in all other laboratory and natural strains tested. Only the former mutation has been proven to be involved in sporulation efficiency, and this same mutation has also been found as causal for a Dobzhansky-Muller incompatibility (Anderson et al. 2010). Although several QTL studies have been performed where MKT1288c was identified as disadvantageous, the definite proof of the pleotropic defect of MKT1S288c allele regardless of the strain background is still lacking. We therefore performed allele swaps of MKT1288c to several strains non-related to S288c. Furthermore, we generated strains with different combinations of single nucleotide variants of MKT1 present in the S. cerevisiae population. We tested the resulting strains for several quantitative phenotypes, elucidating the pleotropic effect of MKT1S288c allele.

Anderson JB, Funt J, Thompson DA, Prabhu S, Socha A, Sirjusingh C, Dettman JR, Parreiras L, Guttman DS, Regev A, Kohn LM (2010) Determinants of divergent adaptation and Dobzhansky-Muller interaction in experimental yeast populations. Curr Biol 20(15): 1383–1388.

Keywords: *pleiotropic gene, quantitative traits, population genetics*

[PS17-16] Reintegration of Extrachromosomal Circular DNA Elements into Chromosomes

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Gene amplification is a known major driver of evolution, playing an important role in the formation of paralogous genes and gene families. The mechanisms behind this process have

been mainly attributed to non-allelic homologous recombination. However, not all gene amplifications can be explained following this model.

We present a model where extrachromosomal circular DNA elements (eccDNAs) are frequent intermediates during genomic rearrangements. We have previously demonstrated that eccDNAs are common in Saccharomyces cerevisiae (1), covering 23% of the genome. Our focus now is to investigate whether eccDNAs reintegrate into chromosomes, which could lead to stable fixation of gene amplifications.

We have previously identified circular elements containing parts from different genes, e.g. the [HXT6/7circle] formed from the hexose transporter genes HXT6 and HXT7 (1). To analyse the dynamics of eccDNA, we studied one copy HXT6 HXT7 yeast populations grown under prolonged glucose limitation. Subpopulations containing [HXT6/7circle] were detected before stable chromosomal amplifications, HXT6 HXT6/7 HXT7, could be detected, suggesting that the [HXT6/7circle] contributed to the amplification of the hexose transporter genes.

We tested eccDNA reintegration into the genome was tested using a different approach. A URA3 marker cassette was inserted in a locus known to form eccDNA. These URA3 strains served as eccDNA donors in crosses with a recipient kar1 ura3 strain. The recipient strain was deficient for karyogamy, allowing transfer of primarily small DNA elements to the receiving nucleus (kar1 strain). We analysed whether eccDNAs had integrated in the recipient genome by restriction enzyme cleavage, followed by DNA ligation, inverse PCR and Sanger sequencing.

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Keywords: *Circular DNA, Gene amplification, Genome evolution*

[PS17-17] Evidence for a Far East Asian origin of Saccharomyces yeasts

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The yeast species in the genus Saccharomyces are used worldwide as microbial agents for baking, fermentation of wine, beer, spirit and other alcoholic beverages. They are also extensively used as model systems in genetics and molecular biology. The genus Saccharomyces is now becoming a super model system in the research of evolutionary biology. However, the geographic distribution and population structure of Saccharomyces species in nature remain elusive. Our recent field survey at an unprecedented scale resulted in the isolation of near 1,000 Saccharomyces strains from different regions of China, covering temperate, subtropical and tropical climate zones. All the currently recognized biological species of the genus have been found from China and different species exhibit different geographic distribution patterns. Population genetics analyses

showed that Chinese isolates of S. cerevisiae contribute the majority of the global variation of the species and contain at least nine surprisingly diverged wild lineages, including the oldest lineages documented so far. Two native lineages including a new one of S. paradoxus coexist in China. S. eubayanus, which is the wild genetic stock of lager yeast firstly found from Patagonia, is actually native to the Tibetan Plateau with three distinct lineages exhibiting over 6% sequence divergence. A Tibetan population of S. eubayanus is more closely related to lager yeast than the Patagonian population which was considered as the wild genetic stock of lager yeast, suggesting that the Tibetan population is the progenitor of lager yeast. In conclusion, Far East Asia appears to be the sole region in the world harboring all the recognized natural species of the genus Saccharomyces and the yeast strains from this region exhibit much higher genetic diversity than those from other regions of the world, suggesting that Far East Asia is likely the origin center of Saccharomyces yeasts.

Keywords: *Saccharomyces, Population, Diversity, Evolution*

[PS17-18] Allodiploid Zygosaccharomyces Genomes Sequencing Assists in Deciphering the Genetic Basis of Hybrid Sterility

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In Saccharomyces cerevisiae diploid MATα/MATa cells, a1-α2 heterodimer encoded by mating-type (MAT) genes is the master sensor of diploidy, promoting the meiosis under starvation, and the repression of haploid-specific genes (h-sgs), such as HO gene, under standard growth conditions. However, how other biotech yeasts govern life cycle remains poorly understood. Halotolerant Zygosaccharomyces rouxii yeasts have applications in food spoilage and fermentation, and exhibit ploidy and karyotype variation, tendency to hybridization, and high diversity in osmostress response. Here, the allodiploid Z. rouxii ATCC42981 serves as a model to investigate how transcriptional network incompatibility affects hybrid sterility.

To extend the Zygosaccharomyces genomic knowledge, we sequenced ATCC42981 and Z. sapae ABT301T genomes. We obtained high-quality assemblies (33-45 scaffolds) by combining heterozygosity reduction and assisting the assembling process with Z. rouxii CBS732T reference chromosomes. Both strains are hybrids derived from one lineage that is over 99% identical to CBS732T and another one not yet identified and ~14% diverging from Z. rouxii type-strain. Interestingly, ATCC42981 and ABT301T differ from one another for the second parent. ATCC42981 kept all reference chromosomes, while ABT301T probably lost one. Both genomes harbor chromosomes from the second parental species, suggesting that unexpectedly no reduction of one of the parental karyotypes occurred after hybridization to resolve genome incompatibility.

In ATCC42981 genome, MATa1 and MATα2 genes are from two different parents (Z. rouxii and Z. sapae, respectively). The different evolutionary history of a1 and α2 subunits could generate negative epistasis accounting for ATCC42981 inability to repress HO gene and to regulate the main meiosis inducer IME4 gene. To prove this, Z. sapae MATα was disrupted by a loxP–kanMX–loxP cassette. Surprisingly, we found that the deletion mutants still actively transcribed MATα, expressed HO and were unable to sporulate by autodiploidization. Besides, MATα removal did not rescue the ability of ATCC42981 to make conjugated asci in mixture with Z. rouxii mating testers. Overall, this work suggests that 1) HO expression does not assure mating-type switching; 2) with 1 copy of MAT disrupted, ATCC42981 does not behave as a haploid; 3) MATα deletion induces HMLα loci de-silencing or, alternatively, reveals the incomplete silencing of donor cassettes in the wild type strain.

Keywords: *Zygosaccharomyces rouxii, Hybrid sterility, Allodiploid genome, a1-α2 heterodimer, Dysregulation of cell-type identity, Mating-type switching, Chimeric sex-determination system*

[PS17-19] Genome-Wide Analysis of Genetic Variation in Flor Yeast Strains

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Flor yeast strains represent a specialized group of yeasts used for centuries in various countries for biological wine aging.

Using a combination of PacBio and Illumina NGS platforms we have sequenced the genomes of three flor strains from culture collection. Comparative genomics approaches were used to uncover the extent of genomic alterations in flor yeast potentially related to their domestication, adaptation and performance in harsh winemaking conditions. SNP analysis versus available genomes of wine and flor strains revealed 2836 genetic variants in 1343 loci specific to flor strains. GO analysis showed that genes associated with ion homeostasis, response to osmotic stress, DNA repair, carbohydrate metabolism, lipid metabolism, cell wall biogenesis, etc., are among the most polymorphic in flor strains.

Pan-genomic analysis discovered multiple events of gene loss including several clustered deletions of asparaginase genes on chromosome XII, FRE-FIT cluster on chromosome XV, MAL gene cluster on chromosome VII, HXT11, four DUP240 genes. Events of gene acquisition were limited to well-known examples of introduction of non-reference genetic material with potential industrial importance, excluding, however, EC1118 regions “A” and “C”.

CNV analysis revealed significant CN-reduction of genes encoding some transmembrane transporters, genes important for septin ring formation and yeast cell budding. Specifically amplified in flor strains were genes for several transporters (HXT6,HXT7, VBA3, MCH2, TPO2), genes involved in stress response (SSA1,GEX1), some unknown proteins.

However, the dominant feature of CN variation in flor strains possibly related to their enhanced ability of velum formation is the alteration of the balance between members of the FLO gene family and other genes for cell-periphery related proteins. While in flor strains the FLO11 gene was amplified several-fold, for other adhesin-encoding genes (FLO1, FLO5,FLO9) we observed either complete loss or significant CN-reduction. We also observed significant CN-reduction of the PAU gene family members, elimination of some of the COS genes.

Our study provides new insights in the nature of genetic variation in flor yeast strains and demonstrates that different adaptive properties of flor yeast strains could have evolved through different mechanisms of genetic variation – point mutations and CN-alteration.

Supported by the Russian Science Foundation grant 16-16-00109.

Keywords: *Flor yeast, Genome, Polymorphism, SNP, Copy number variation*

[PS17-20] The Role of the S. cerevisiae Sco2p and Its Homologues in Antioxidant Defense and Redox Homeostasis

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Mitochondria are the “powerhouse” of the cell that generate the major part of cellular energy by oxidative phosphorylation. Cytochrome c oxidase (COX) is an essential enzyme of this process by transfering electrons from cytochrome c to molecular oxygen.

The Sco proteins, present in all kind of organisms, are regarded as one of the key players in the COX assembly. Many eukaryotes including Saccharomyces (S.) cerevisiae and human possess two distinct SCO genes with a high degree of sequence similarity. So far, functional studies and phylogenetic analyses have revealed multiple roles of Sco proteins including copper homeostasis and redox signaling. Moreover, the presence of a thioredoxin fold and thus a structural similarity to antioxidant enzymes (including peroxiredoxins and thiol-disulfide oxidoreductases) hint at an (additional) antioxidant function for these proteins.

Our current studies in S. cerevisiae revealed a possible role of Sco2 protein (ScSco2p) in antioxidant defense mechanisms. Since S. cerevisiae is a good model to functionally characterize genes from more complex organisms, complementation studies with SCO-homologs from different organisms (Homo sapiens, Schizosaccharomyces pombe, Arabidopsis thaliana, Drosophila melanogaster and Kluyveromyces lactis) were carried out to test whether they are able to substitute for the function of ScSco2p. The observation of functional as well as non-functional homologs allows us to identify aminoacid(s) that are crucial for the antioxidant function. Moreover, the pathogenicity of reported mutations of the human SCO homologs was characterized with regard to oxidative stress.

Keywords: *Sco, antioxidant defense, model organism, homolog*

[PS17-21] The Evolution of Pheromone Diversity in Fission Yeast

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New species emerge when two populations can no longer interbreed. Reproductive isolation is a key process of speciation. In yeasts, mate selection critically depends on the molecular recognition of two peptidyl mating pheromones by their receptors. Therefore, pheromone diversity is important to promote speciation in yeasts. Recent studies have revealed that only a few mutations in a pheromone and its receptor can give rise to a new species via the process of reproductive isolation in the fission yeast Schizosaccharomyces pombe (Seike et al., PNAS, 2015). The mechanism of ongoing speciation in nature through mutations in pheromones, however, remains to be elucidated.

S. pombe has two mating types, Plus (P) and Minus (M). P- and M-cells secrete two different mating pheromones (P-factor and M-factor) during mating. In this study, we collected 150 S. pombe strains whose origin differs from the laboratory strain L968, and sequenced their pheromone genes. Interestingly, the primary sequence of M-factor was completely conserved, whereas that of P-factor was very diverse. In the L968 strain, P-factor gene encodes four tandem copies of pheromone ORFs, but there were some strains in which copy number of P-factor increased (5–8 copies). In addition, we analyzed the recognition specificities of pheromones/receptors between two fission yeasts, S. pombe (Sp) and the related species S. octosporus (So). Three of nine residues in the M-factor were different between two species, and the So-M-factor was not functional in S. pombe. In contrast, P-factors are very different between two species. Nevertheless, the So-P-factors were partially functional in S. pombe allowing these cells to mate successfully using So-P-factors. Thus, recognition specificity of the M-factor is tight whereas that of the P-factor is loose. Provably, the biased system for pheromone recognition allows flexible adaptation in yeasts to mutational changes in a combination of pheromones/receptors while maintaining tight recognition for mating partners.

Keywords: *fission yeast, mating pheromone, evolution*

[PS17-22] Experimental search for factors influencing rate of gene evolution

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There are several hypotheses postulating that gene function is not the only factor influencing the rate of sequence evolution. The assumptions are supported by evidence that expression of genetic information is optimized on many levels from sequence, through transcription and translation to protein function, life and utilization. Natural gene duplicates (paralogous genes) in yeast genome give an a potential to test recently hypothesized relationship between expression levels of genes and rates of their evolution. Data collected in our experiments also shed the light on the relevance of several other

factors influencing evolutionary rate.The collection of 628 strains (314 paralogous pairs) with single genes controlled by the GAL promotor was created. The fitness cost of overexpression was estimated for each paralog in two experimental regimes: (1) maximum growth rate and (2) direct competition. Both tests were performed in several environmental conditions to broaden the spectrum of detected protein toxicity. We show some preliminary comparisons between differently expressed paralogs as well as results for the whole pool of constructed strains.

Keywords: *gene expression level, rate of evolution, paralogs*

[PS17-23] Comparative Genomics of Pichia kudriavzevii and Candida krusei

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UCD (Ireland)

Candida krusei is one of the five most prevalent yeasts in the Candida pathogenic species complex, which is responsible for significant levels of morbidity and mortality in immunocompromised patients. Pichia kudravzevii is an environmental yeast that has a major role in biotechnology and is not regarded as pathogenic. The two yeasts have previously been regarded as separate species, but recent studies suggest that they are the same species, with Candida krusei being a pathogenic, non-mating form of Pichia kudriavzevii. However, no complete genome has been sequenced and no analyses of genomics, pathogenicity or mating between the two have been performed. As P. kudriavzevii has a biotechnological role, it is important for biosafety concerns to clarify the relationship between P. kudriavzevii and C. krusei strains.

We generated and annotated a reference genome sequence for a clinical isolate of C. krusei using a combination of PacBio and Illumina sequencing. The genome is 10.8 Mb, organized into 5 chromosomes. It has unusual centromere stuctures consisting of large inverted repeats. We conducted a comparative analysis of Illumina data from 32 worldwide isolates of C. krusei and P. kudriavzevii. SNPs from 167,000 variable sites were used to generate a phylogenetic tree of isolates, which shows little geographical structure and no clear separation between clinical (C. krusei) and environmental (P. kudriavzevii) isolates, suggesting that infections may originate from the environment. Most isolates are diploid and heterozygous, but several examples of apparent triploidy, trisomy and extensive losses of heterozygosity were identified. Isolates with high drug resistance were found from environmental as well as clinical sources.

Phylogenetic analysis shows that C. krusei is only distantly related to other Candida species. It lies within the genus Pichia, and is more closely related to methylotrophic yeasts such as Ogataea polymorpha than to C. albicans or C. glabrata.

Keywords: *Candida krusei, Pichia kudriavzevii, Comparative Genomics, Bioinformatics*

[PS17-24] Adaptive Laboratory Evolution of Saccharomyces cerevisiae Diploid Strains for Mannitol Utilization as a Carbon Source

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Crude oil reserves are becoming increasingly scarce, and biorefinery systems that integrate biomass conversion processes and equipment to produce fuels, power, and chemicals from annually renewable resources are a promising technology to move away from a petroleum-based society to a biomass-based society. One interesting biomass that has not been extensively utilized is marine biomass such as brown macroalgae (kelp). The composition of brown macroalgae includes up to 55% dry weight of the carbohydrates laminarin, mannitol and alginate, and it does not contain lignin. Hence, macroalgae are a very promising feedstock for microbial conversion of all carbohydrates into biofuels and valuable chemicals. Despite the presence of this native catabolic pathway, many yeast strains cannot catabolize mannitol or require adaptation to do so.

In this study a screening of thirty six strains, isolated from different sources, was performed. The strains were grown on complex and minimal media with mannitol as a main carbon source. Fifteen strains showed growth on complex media-mannitol (CM-mannitol) and just three diploid strains were capable to growth on minimal media-mannitol (MM-mannitol). After a couple of months of Adaptive Laboratory Evolution (ALE) three Saccharomyces cerevisiae diploid strains (YPS606, RM11 and T7) were successfully adapted to grow on MM-mannitol. Despite the efforts, the laboratory CENPK113-7D strain was unable to utilize this sugar alcohol as a carbon source.

Keywords: *Adaptive Laboratory Evolution, Diploid Strains, Mannitol*

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