EDITORIAL

We are delighted to welcome the worldwide yeast community in Gothenburg for ICYGMB2019!

The “International Yeast Conferences” started in the 1960s with a handful of delegates and since then have become THE most important event in yeast research. Now the yeast meeting to returns to Gothenburg. Many yeast researchers still remember the meeting in 2003 with over 1,100 delegates, a truly memorable event.

The Life Sciences are changing, and yeast research remains at their forefront. Advancements in genome sequencing and genome editing just make yeast more exciting as model organism in basic cell biological research, genome evolution and as a tool for synthetic biology and biotechnology.

One of the most important reasons for the enormous success of yeast research lies in the unique character of the international yeast research community. No other community employs such a free exchange and access to information and research tools. Nor has any other community had the ability to build – even intercontinental – consortia of critical mass to tackle large-scale projects, such as in sequencing the first eukaryotic genome or the first comprehensive yeast knockout library. Yeast2019 is the meeting of the international yeast research community where the latest, and even unpublished results are exchanged, and new projects, alliances, and collaborations are founded. A do-not-miss-event.

We attempt to incorporate the present excitement in yeast research in the programme of yeast2019. We are confident that this conference will contain important news and information for all yeast researchers. Taken together, yeast2019 will provide an up-to-date overview in yeast research and it will set the scene for years to come. After all, it is one of the most affordable international conferences of such as size and reputation!

On behalf of the organising committee,

Stefan HOHMANN, chair ICYGMB2019 Gothenburg

Committees

STEERING COMMITTEE

Stefan Hohmann, chairman
Terrance G. Cooper, secretary
Jiří Hašek , chair of yeast2017

FINANCE AND POLICY COMMITTEE OF THE YEAST RESEARCH COMMUNITY

Australia: **Ian W. Dawes**
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Belgium: **Johan Thevelein**
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Spain: **Andres Aguilera**
Sweden: **Stefan Hohmann**
Switzerland: **Claudio De Virgilio**
Tunisia: **Omrane Belhadj**
Ukraine: **Andrei Sibirny**
United Kingdom: **Stephen G. Oliver**
United States: **Terrance G. Cooper**

LOCAL ORGANISING COMMITTEE

From **Chalmers University of Technology**: Stefan Hohmann (Chair) Jens Nielsen, Dina Petranovic, Lisbeth Olsson, Carl Johan Franzén, Cecilia Gejer, Mikael Molin, Florian David, Verena Siewers, Kate Campbell, Christer Larsson, Joakim Norbeck, Thomas Andlid

From the **University of Gothenburg**: Anders Blomberg, Thomas Nyström, Per Sunnerhagen, Claes Gustafsson, Markus Tamás, Beidong Liu, Jonas Warringer, Marija Cvijovic

From the **Umeå University**: Stefan Björklund

EXECUTIVE COMMITTEE

Stefan HOHMANN (Chair), Mikael MOLIN (secretary), Jonas WARRINGER, Florian DAVID, Verena SIEWERS, Anders BLOMBERG, Per SUNNERHAGEN, Marija CVIJOVIC

Practical information

ORAL PRESENTATIONS

Presentations should be uploaded to the conference computers before the respective session. In each room both PC and MAC computers are available.

POSTER PRESENTATIONS

Poster numbers are corresponding to your submission number. Please mount your posters on Sunday.
Schedule for poster presentations:
Monday: even posters
Tuesday (lunch): odd poster
Tuesday (evening): all posters
Wednesday: all posters

Three Poster prizes will be announced during the conference dinner

SPEAKERS' CORNER

All **keynote and plenary speakers** are kindly invited to join the speakers´ corner where conference participants will have the possibility to meet them. Each speaker will have a volunteer assigned that will help them find their way. Speakers should be present during the speakers´ corner session that directly follows their presentation:

Speakers presenting Sunday or Monday morning: speakers´ corner Monday 14.00-16.00
Speakers presenting Monday afternoon or Tuesday morning: speakers´ corner Tuesday 14.00-16.00
Speakers presenting Tuesday afternoon or Wednesday morning: speakers´ corner Wednesday 14.00-16.00
Speakers presenting Wednesday afternoon or Thursday: speakers´ corner Thursday 13.00-14.00

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Opening keynote lectures

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VÄSTRA GÖTALANDSREGIONEN

Poster prize

FEMS YEAST RESEARCH

MGG Molecular Genetics and Genomics

Other

SUNRISE SCIENCE PRODUCTS

G3 Genes | Genomes | Genetics

Program Overview

SUNDAY AUGUST 18

	Main auditorium F1+F2	F4	F5
12:00	Registration; poster mounting		
16:00	Opening ceremony		
16:30	**Frederick ROTH,** Toronto, Canada		
17:30	**Roger KORNBERG,** Stanford, USA		
18:30	Welcome reception		

MONDAY AUGUST 19

08:30	Sensing, signaling and stress responses		
10:30	Coffee break		
11:00	Stress signalling and (protein) trafficking	Modern yeast biotechnology	Controlling gene expression
13:00	Lunch, Poster session, Speaker's corner, SGD Q&A , Exhibitions, Coffee		
16:00	Gene expression, RNA processing and regulation		
18:00	ICYGMB special lecture: Steve OLIVER		

TUESDAY AUGUST 20

08:30	Metabolism and metabolic regulation		
10:30	Coffee break		
11:00	Metabolism and organelles	RNA biology	Cell growth, division and morphogenesis
13:00	Lunch, Poster session, Speaker's corner, SGD Q&A, Exhibitions, Coffee Meeting of the Finance and Policy Committee of the International Yeast Community (members - on invitation only): 1300-1500 room R13		
16:00	Biotechnology and synthetic biology		
18:00	Poster session and social activities (live music, bar)		

WEDNESDAY AUGUST 21

08:30	Evolution and population genetics		
10:30	Coffee break		
11:00	Pathogens and host interaction	Ageing and disease models	Population, comparative and evolutionary genomics
13:00	Lunch, Poster session, Speaker's corner, Exhibitions, Coffee, SGD Workshop		
16:00	Cell cycle and cell fate		
19:00	Conference dinner		

THURSDAY AUGUST 22

08:30	Quality control, organelles and aging		
10:30	Coffee break		
11:00	Yeast molecular networks	Proteostasis	Heterologous gene function and drug discovery
13:00	Lunch, Speaker's corner, SGD Q&A , Coffee		
14:00	DNA replication, mutation and repair		
16:00	**Susan GASSER,** Basel, Switzerland		
17:00	Closing ceremony		

Detailed Program

XXIX INTERNATIONAL CONFERENCE ON YEAST GENETICS AND MOLECULAR BIOLOGY

ICYGMB 2019

Göteborg (Gothenburg) Sweden August 18–22, 2019

SUNDAY AUGUST 18

	Main auditorium F1+F2	F4	F5
12:00	Registration; poster mounting		
16:00	Opening ceremony		
16:30	Chair: Kenneth Wolfe **Frederick ROTH,** University of Toronto, Canada *Using yeast to map environment-dependent protein networks and functional human sequence variation*		
17:30	Chair: Jean Beggs **Roger Kornberg,** Stanford University, USA *Chromosome Structure and Transcription*		
18:30	Welcome reception		

MONDAY AUGUST 19

	Main auditorium F1+F2	F4	F5
08:30	Chair: Stephen Oliver **Sensing, signaling and stress responses** **Francesc POSAS,** Institute for Research in Biomedicine (IRB Barcelona), Spain 08:30-09:00 *Adaptation to Osmostress by the HOG1 SAPK* **Symeon SINNIOSSOGLOU,** University of Cambridge, UK 09:00-09:30 *Compartmentalized Synthesis of Triacylglycerol Regulates Nuclear Membrane Homeostasis During Stress* **Peter SWAIN,** University of Edinburgh, UK 09:30-10:00 *Cellular decision-making in changing environments* **Martha CYERT,** Stanford University, USA 10:00-10:30 *Networks for the Calcineurin Phosphatase in Yeast and Humans Reveal Evolution of Signaling*		
10:30	Coffee break		
11:00	**Stress signalling and (protein) trafficking** Chairs: Mikael Molin and Claudio De Virgilio **11.00 Sabine ROSPERT,** University of Freiburg, Germany *The Hsp70 Homolog Ssb: a novel Component of the TORC1/Sch9 Signaling Network* **11.15 Julie PARENTEAU,** Université de Sherbrooke, Canada *Mechanisms of Intron Mediated Nutrients Sensing and Resistance to Starvation* **11.30 Riko HATEKEYAMA,** Université de Fribourg, Switzerland *Emerging Links Between TORC1 Signaling And Intracellular Trafficking*	**Modern yeast biotechnology** Chairs: Florian David and Petri-Jaan Lahtvee **11.00 Filipa PEREIRA,** EMBL Heidelberg, Germany *In silico Yeast Chassis Design for Accelerated Cell Factory Development* **11.15 Florian DAVID,** Chalmers, Sweden *Framework for Model Assisted Generation of Platform Strains through CRISPR Mediated Fine-tuning of Central Carbon Metabolism*	**Controlling gene expression** Chairs: Stefan Björklund and David Shore **11.00 Shira URIM,** Israel Institute of Technology, Israel *"mRNA Imprinting": Regulation of Gene Expression by Co-transcriptionally Associating Proteins* **11.15 Nozomu SAEKI,** Okayama University, Japan *Systematic Identification of Genes whose Overexpression Positively Affect Fitness in Various Environments*

11.45 Silvia SOTO DIAZ, Université Libre de Bruxelles, Belgium
Characterization of theactivity regulation of Mep-Amt-Rh ammonium transport proteins by TORC1-Npr1-Amu1/Par32 in Saccharomyces cerevisiae

11.52 Allyson O'DONNELL, University of Pittsburgh, USA
Fluorogen Activating Proteins as a Powerful New Imaging Tool for Quantitative Protein Trafficking Studies in Yeast

12.00 Serge PELET, Université de Lausanne, Switzerland
Correlation of MAPK Activity and Gene Expression in Single Cells

12.15 Kyle CUNNINGHAM, Johns Hopkins University, USA
Regulation of Ceramide Synthase and CK2 by Calcineurin During ER stress

12.30 Mikael MOLIN, Chalmers, Sweden
Peroxiredoxin promotes longevity and H_2O_2-resistance in yeast through redox-modulation of protein kinase A-dependent nutrient signalling

12.45 Dieter SAMYN, Swedish University of Agricultural Sciences, Sweden
ILF and VRED Pathways Prevent Vacuole/Lysosome Rupture by Oxidative Stress

12.52 Michael SCHWEIZER, Heriot-Watt University, United Kingdom
Evidence for Separation of PRPP Production and Maintenance of Cell Wall Integrity: Two Essential Functions of the PRPP-synthesising Machinery in Saccharomyces cerevisiae

11.30 Thomas NICOLAÏ, KU Leuven, Belgium
Production Of Muconic Acid And Protocatechuic Acid From Lignocellulosic Feedstock

11.45 Tomas STRUCKO, Danish University of Technology, Lyngby, Denmark
A Universal Tool for Identification of Superior Cell-factory Hosts in Genetically Diverse Yeast Species

12.00 Hannah BLITZBLAU, Novogy, Inc., Cambridge, USA
Production of Branched Fatty Acids in Yarrowia lipolytica

12.15Laura NAVONE, Queensland University of Technology, Australia
Insights into protein production bottlenecks in Pichia pastoris: complexity of expressing thermostable phytases

12.30 Yongjin ZHOU, Dalian Institute of Chemical Physics, China
Precise Genome editing in Methylotrophic Yeast by Enhancing Homologous Recombination

12.45 Petri-Jaan LAHTVEE, University of Tartu, Estonia
3D Printing of Yeast-Laden Hydrogels for Improved Bioprocess Efficiencies

11.30 Asli AZIZOGLU, ETH Zürich, Switzerland
The Well-Tempered Controller-846: A Precisely Titratable and Tight Transcriptional Controller in S. cerevisiae

11.45 Anna BABOUR, INSERM, France
The Chromatin Remodeler Isw1Is A Novel Actor Of The Unfolded Protein Response

12.00 Mónica PAVÒN VERGÉS, Universidad Complutense de Madrid, Spain
The SAGA/TREX-2 Subunit Sus1 Plays a Significant Role in the Adaptive Transcriptional Response Mediated Through the CWI Pathway in Saccharomyces cerevisiae

12.15 Brandon HO, University of Toronto, Canada
Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae

12.30 Albert SERRA-CARDONA, Columbia University, USA
Mechanisms Underlying the Distribution of Parental and Newly Synthesized H3-H4 Histones during DNA Replication

12.45 Martin KUPIEC, Tel Aviv University, Israel
The Many Roles of Elg1 in the Maintenance of Genome Stability and Chromatin States

13:00 Lunch, Poster session (even numbers), Speaker's corner, SGD Q&A, Exhibitions, Coffee

MONDAY AUGUST 19

	Main auditorium F1+F2	F4	F5
16:00	**Gene expression, RNA processing and regulation** Chair: Stefan Björklund **Katja STRÄSSER,** Justus Leibig University, Germany 16:00-16:30 *Nuclear mRNP Assembly and Export* **Jean BEGGS,** University of Edinburgh, UK 16:30-17:00 *Crosstalk between transcription, splicing and chromatin* **David SHORE,** Université de Genève, Switzerland 17:00-17:30 *A rapid and highly specific transcriptional response to ribosome assembly stress helps to maintain protein homeostasis*		
17:30	**ICYGMB special lecture** Chair: Stefan Hohmann **Stephen OLIVER,** University of Cambridge, UK 17:30-18:00 *Using yeast to model human diseases: parasites to paralysis*		

TUESDAY AUGUST 20

	Main auditorium F1+F2	F4	F5
08:30	**Metabolism and metabolic regulation** Chair: Symeon Sinniossoglou **Maitreya DUNHAM,** University of Washington, USA 08:30-09:00 *High throughput mutagenesis strategies to understand enzyme function and predict disease risk* **Markus RALSER,** University of Cambridge, UK 09:00-09:30 *Metabolite exchange interactions between yeast cells, and how they create cellular heterogeneity* **Andreas MAYER,** Université de Lausanne, Switzerland 09:30-10:00 *Intracellular phosphate reception and signaling: A novel homeostatic system with roles for an "orphan" organelle?* **Merja PENTTILÄ,** VTT, Finland 10:00-10:30 *Engineering Yeasts for Production of Glycolic Acid, a monomer for bioplastic PGA*		
10:30	Coffee break		
11:00	**Metabolism and organelles** Chairs: Verena Siewers and Ida van der Klei **11.00 Lieselotte VERMEERSCH,** VIB-KU Leuven, Belgium *Natural Variation and Heterogeneity in the S. cerevisiae Lag Phase* **11.15 Mira ROSENTHAL,** Weizmann Institute of Sciences, Israel *Uncovering Targeting Priority to Peroxisomes using a new Targeting Competition Assay*	**RNA biology** Chairs: Per Sunnerhagen and Tracy Nissan **11.00 Ruchika SACHDEV,** ETH Zürich, Switzerland *Regulating the Dynamicity of Stress Induced Cytoplasmic Ribonucleoprotein Granules via Liquid-Liquid Phase Separation* **11.15 Vicent PELECHANO,** Karolinska Institute, Sweden *Transcriptional Complexity and Functional Consequences of Cryptic Promoters*	**Cell growth, division and morphogenesis** Chairs: Kate Campbell and Jan Skotheim **11.00 Attila CSIKÁSZ-NAGY,** King's College London / Pázmány Péter Catholic University, UK/Hungary *Measuring and Modelling Yeast Colony Growth* **11.14 Ulrike MÜNZNER,** Kyoto University, Japan *A Mechanistically Detailed Model of the Cell Division Cycle in Saccharomyces cerevisiae*

11.30 Helen CAUSTON, Columbia University, USA
Understanding the Temporal Regulation of Metabolism in Yeast

11.45 Barry BOCHNER, Biolog Inc., USA
New Mitochondrial Function Assays Demonstrate Substantial Differences among Yeasts and Uncover Novel Metabolic Energy Pathways

12.00 Elizabeth WALDEN, University of Ottawa, Canada
Phenomic Screen Implicates the Yeast Lysine Acetyltransferase NuA4 in Regulation of Glycogen Synthesis and Mitochondrial Morphology through the PKA Inhibitor Bcy1

12.15 Łukasz SYGA, University of Gothenburg, Sweden
Diffusion and Localization of Proteins in the Plasma Membrane of Saccharomyces cerevisiae

12.30 Ida VAN DER KLEI, University of Groningen, The Netherlands
Identification and Function of Novel Peroxisome Contact Sites in the Yeast Hansenula polymorpha

11.30 Igor KUKHTEVICH, Helmholtz Zentrum München, Germany
A Novel Method to Follow RNA Expression in Live Yeast Cells at the Single-Cell Level Over the Cell Cycle and Multiple Generations

11.45 Hanna ALALAM, University of Gothenburg, Sweden
Genetic Screening for New Factors Affecting NMD in S. cerevisiae

12.00 Yulia VASIANOVICH, Université de Sherbrooke, Canada
Tracking Telomerase RNA via Inducible Tagging

12.15 Alon APPLEBOIM, Hebrew University of Jerusalem, Israel
mRNA Homeostasis Perturbed: Rapid Depletion of Chromatin and RNA Processing Factors Reveals the Limits of mRNA Homeostasis

12.30 Kathryn TURNBULL, Umeå University, Sweden
A role for the Saccharomyces cerevisiae ABCF protein New1 during translation termination

12.45 Frank ALBERT, University of Minnesota, USA
Simultaneous Quantification of mRNA and Protein in Single Cells Reveals Transacting Genetic Variation

11.28 Kurt SCHMOLLER, Helmholtz Zentrum München, Germany
Template-limited Transcription Coordinates Histone Homeostasis with DNA Content

11.42 Kate CAMPBELL, Chalmers, Sweden
Metabolism is Tailored to Biosynthetic Needs During the Budding Yeast Cell Cycle

11.56 Aleksandar VJESTICA, Université de Lausanne, Switzerland
Coordinate regulation of the cell cycle and re-fertilization blocks ensures ploidy maintenance in fission yeast

12.10 COFFEE BREAK

12.18 Ines DE OYA, CABIMER / CSIC - University of Seville - University Pablo de Olavide, Spain
A Novel Connection Between the Nuclear Pore Complex and Cell Cycle Regulation

12.32 Jan SKOTHEIM, Stanford University, USA
The biosynthetic basis of cell size control

12.46 Deniz IRVALI, University of Tuebingen, Germany
Start is not the Metabolic Commitment Point of the Yeast Cell Cycle

13:00 Lunch, Poster session (odd numbers), Speaker's corner, SGD Q&A, Exhibitions, Coffee

Meeting of the Finance and Policy Committee of the International Yeast Community (members - on invitation only): 1300-1500 room R13

TUESDAY AUGUST 20

	Main auditorium F1+F2	F4	F5
16:00	**Biotechnology and synthetic biology** Chair: Franscesc Posas **Zhongjun QIN,** Shanghai Institute of Plant Physiology and Ecology, China 16:00-16:30 *Creating a functional single chromosome yeast* **Neel S JOSHI,** Harvard University, USA 16:30-17:00 *Solar Panels On Yeast: Inorganic Biohybrids For Enhanced Photochemical Production* **Eckhard BOLES,** Goethe-University, Germany 17:00-17:30 *Two Different Strategies to Engineer Yeast for Production of Aromatic Compounds* **Jens NIELSEN, Chalmers, Sweden** 17:30-18:00 *Synthetic Biology of Yeast*		
18:00	Poster session (all posters) and social activities (live music, bar)		

WEDNESDAY AUGUST 21

	Main auditorium F1+F2	F4	F5
08:30	**Evolution and population genetics** Chair: Maitreya Dunham **Daniel JAROSZ,** Stanford University, USA 08:30-09:00 *Mapping drivers of phenotypic change at single nucleotide resolution* **Judith BERMAN,** Tel Aviv University, Israel 09:00-09:30 *Antifungal drug responses of cells and populations* **Daniela DELNERI**, University of Manchester, UK 09:30-10:00 *Functional profiling of inter-genic and intronic non coding RNAs* **Kenneth WOLFE,** University College Dublin, Ireland 10:00-10:30 *Multiple reinventions of mating-type switching during budding yeast evolution*		
10:30	Coffee break		
11:00	**Pathogens and host interaction** Chairs: Karl Kuchler and Per Ljungdahl **11.00 Alexander LORENZ,** University of Aberdeen, UK *Genome Stability, Karyotype Plasticity, DNA Damage Response, and Morphogenetic Switching in the Fungal Pathogen Candida auris* **11.12 Sabrina JENULL,** University of Vienna, Austria *The Candida albicans HIR Histone Chaperone Complex is a Novel Player in Fungal Virulence*	**Ageing and disease models** Chairs: Marija Cvijovic and Tiago Outeiro **11.00 Alexander ALEXANDROV,** Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Russia *Distinct patterns of cell death and division arrest at early replicative ages revealed by genome-wide screening* **11.15 Alexander DELUNA,** Centro de Investigación y de Estudios Avanzados de IPN, Mexico *Cellular mechanisms of longevity by dietary restriction revealed by large-scale competitive-aging screens*	**Population, comparative and evolutionary genomics** Chairs: Jonas Warringer and Daniela Delneri **11.00 Fabio PUDDU,** University of Cambridge, UK *Exploring genome architecture and stability in the absence of any single gene* **11.15 Xiangwei HE,** Zhejiang University, China *Reversible Microhomology-Mediated Short Segment Duplication (MHSSD) Generates High Genetic Divergence* **11.30 Matteo de Chiara,** Université de Nice, France *Domestication Reprogrammed The Budding Yeast Life Cycle*

11.24 Chung-Yu LAN, National Tsing Hua University, Taiwan
The Transcription Factor Sfp1 Regulates the Oxidative Stress Response in Candida albicans

11.36 Rosana ALVES, University of Minho, Portugal
Functional Characterization of Putative Acetate Transporters and Channels in the Human Fungal Pathogen Candida glabrata

11.48 Romain LAURIAN, Université Claude Bernard Lyon 1, France
Hexokinase and Glucokinases Are Essential for Fitness and Virulence in the Pathogenic Yeast Candida albicans

12.00 Fitz Gerald SILAO, Stockholm University, Sweden
Candida albicans glutamate dehydrogenase (GDH2) catalyzes environmental alkalization but is dispensable for survival and escape from macrophages

12.12 Yinhe MAO, Institut Pasteur Shanghai, China
The posttranscriptional modification of a conserved iron repressor defines an unexpected detoxification role in Candida albicans gastrointestinal commensalism

11.30 Sara MAVROVA, European Research Institute for the Biology of Ageing, Netherlands
Loss of Physicochemical Homeostasis in Yeast Replicative Ageing

11.45 Iñigo PRADA-LUENGO, University of Copenhagen, Denmark
Global Sequencing of Circular DNA Reveals Loss of Genetic Variation and the Role of Replication Origins for Maintenance of Circular DNA in Aging Yeast Cells

12.00 Carole LINSTER, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg
Nicotinamide Nucleotide Damage and Repair in Yeast

12.15 Benjamin BARRE, IRCAN, France
Intragenic Repeat Expansions Control Yeast Chronological Aging

12.30 Per WIDLUND, University of Gothenburg, Sweden
Variation in Processing of Misfolding Proteins

12.45 Tiago OUTEIRO, University Medical Center Goettingen, Germany
Insights into the molecular underpinnings of Parkinson's disease: lessons from yeast

11.45 Jana HELSEN, KU Leuven, Belgium
Evolutionary Trajectories Reflect the Modularity in Genetic Networks

12.00 Taraneh ZARIN, University of Toronto, Canada
Proteome-wide Signatures of Function in Highly Diverged Intrinsically Disordered Regions

12.15 Abigail KELLER, University of Wisconsin, USA
Identifying Driver Genes Responsible for Condition Dependent Fitness Effects of Synthetic Chromosome Amplifications

12.30 Zoltán FARKAS, Biological Research Centre, Hungary
Compensatory Mutations Drive Morphological Evolution

12.45 Simon STENBERG, University of Gothenburg, Sweden
Superoxide Induces Adaptive Editing of Mitochondrial DNA

WEDNESDAY AUGUST 21

	Main auditorium F1+F2	F4	F5
	12.24 Amandine BONNET, Université de Paris, France *Multiple Functions Of The Nuclear Pore Complex In The Replication Cycle Of The Ty1 Retrotransposon* **12.36 Duccio CAVALIERI,** University of Florence, Italy *Saccharomyces cerevisiae and Social Wasps as Models for the Evolution of Host Microbe Interactions* **12.48 Jürgen WENDLAND,** Hochschule Geisenheim University, Germany *Saccharomycopsis Yeasts: Omics Insight Into These Unique Fungal Predators*		
13:00	Lunch, Poster session (all posters), Speaker's corner, Exhibitions, Coffee		
	SGD Workshop 13:30-15:30		

WEDNESDAY AUGUST 21

	Main auditorium F1+F2	F4	F5
16:00	**Cell cycle and cell fate** Chair: Martha Cyert **Folkert VAN WERVEN,** The Francis Crick Institute, UK 16:00-16:30 *Cell fate control by an alternative transcriptome* **Manuel MENDOZA,** IGBMC Strasbourg, France 16:30-17:00 *Control of cell identity by Nuclear Pore Complex acetylation* **Marti ALDEA,** CSIC Barcelona, Spain 17:00-17:30 *Holistic control by molecular networks in proliferation and aging* **Matteo BARBERIS,** University of Surrey, UK 17:30-18:00 *A Novel and Robust Molecular Design Synchronizing Transcriptional with Cell Cycle Oscillators*		
19:00	Conference dinner		

THURSDAY AUGUST 22

	Main auditorium F1+F2	F4	F5
08:30	**Quality control, organelles and aging** Chair: Markus Ralser **Thomas NYSTRÖM,** University of Gothenburg, Sweden 08:30-09:00 *Identifying new players involved in spatial sequestration of protein aggregates* **Simon ALBERTI**, Max-Planck Institute Dresden, Germany 09:00-09:30 *Molecular mechanisms underlying stress granule formation, function and disease* **Agnieszka CHACINSKA,** University of Warsaw, Poland 09:30-10:00 *Stress responses to mitochondrial dysfunction* **Gilles CHARVIN,** IGBMC, Strasbourg, France 10:00-10:30 *Single cell analysis of entry into replicative senescence: do budding yeast really age?*		
10:30	Coffee break		

THURSDAY AUGUST 22

11:00

Main auditorium F1+F2

Yeast molecular networks

Chairs: Anders Blomberg and Brenda Andrews

11.00 Introduction

11.02 Christoph BÖRLIN, Chalmers, Sweden
Understanding Transcriptional Regulation Of Amino Acid Metabolism Through Mapping Of Transcription Factor Binding Sites Using ChIP-exo

11.18 Gwenael RABUT, CNRS UMR6290, France
Mapping the interaction network of ubiquitylation enzymes in living cells

11.34 Brenda ANDREWS, University of Toronto, Canada
From Phenotypes to Pathways: Global Analysis of Subcellular Compartment Morphology using Systematic Genetics and Single Cell Imaging

11.50 Jolanda VAN LEEUWEN, Université de Lausanne, Switzerland
Systematic analysis of bypass suppressors of essential genes

12.06 Hector GARCIA SEISDEDOS, Weizmann Institute of Science, Israel
Proteins Evolve on the Edge of Supramolecular Self-assembly

12.22 Vaskar MUKHERJEE, University of Gothenburg, Sweden
Phenomics, transcriptomics and metabolomics for identifying concentration-dependent chemical interactions and understanding the mechanistic basis of the mixture toxicity

F4

Proteostasis

Chairs: Beidong Liu and Simon Alberti

11.00 Beidong LIU, University of Gothenburg, Sweden
A stress granule formation pathway identified from yeast imaging-based phenomic screens

11.15 Ivana MALCOVA, Institute of Microbiology of the CAS, Czech Republic
Destabilization of EIF3a Renders the EIF3 Factor Vulnerable to Aggregation at Physiological Growth Conditions and Leads to an Early Formation of Stress Granules in Saccharomyces cerevisiae Cells

11.30 Asim SENGOR, ETH Zürich, Switzerland
Cytoplasmic age-dependent protein aggregates control DNA-circle accumulation in the aging mother cells in S. cerevisiae

11.45 Georgios KARRAS, The University of Texas MD Anderson Cancer Center, USA
Multi-omics dissection of Hsp90-mediated trait plasticity

12.00 Stefanie ANDERSSON, University of Gothenburg, Sweden
Genome-wide Imaging Screen Uncovers Molecular Determinants of Arsenite-induced Protein Aggregation and Toxicity

12.15 Kara SCHNEIDER, University of Gothenburg, Sweden
A High-Content Microscopy-Based Screen for Factors Involved in Aggregate Deposition at Mitochondria

12.30 Tony HAZBUN, Purdue University, USA
Elucidating the function of alpha-N-terminal protein methylation of Hsp31 and other N-terminal methyltransferase substrates in proteostasis and stress response

F5

Heterologous gene function and drug discovery

Chairs: Per Sunnerhagen and Elizabeth Bilsland

11.00 Piotr SOCZEWKA, Polish Academy of Science, Poland
Using Yeast Chorea--acanthocytosis Model for Drug Screening

11.15 Ludimila ALMEIDA, University of Campinas, Brazil
Identification of FUS and OPTN Disaggregating Compounds and their Plasma Membrane Import Routes Using Yeast Based High-content Screening

11.30 Anmoldeep RANDHAWA, International Centre for Genetic Engineering and Biotechnology, India
Fungal Fludioxonil Sensitivity is Relieved by Overexpression of CORVET Complex Components

11.45 Kathy PARISI, La Trobe University, Australia
Novel Insights in the Mode of Action of Diverse Antifungal Plant Defensins from High Throughput Screens of the S. cerevisiae Non-Essential Gene Deletion Collection

12.00 Ward VANTHIENEN, KU Leuven, Belgium
Isolation and Identification of a Novel Class of Oncostatic Glucose Transport Inhibitors Using the Yeast tps1Δ Mutant

12.15 Julia Maria CORONAS-SERNA, Universidad Complutense de Madrid, Spain
Saccharomyces cerevisiae as a Model to Study Supramolecular Protein Complexes in Innate Immunity TLR Signaling

12.30 Agnès MICHEL, University of Oxford, UK
Saturated Transposon Analysis in Yeast goes further

THURSDAY AUGUST 22

	Main auditorium F1+F2	F4	F5
	12.38 Kevin MERCURIO, University of Ottawa, Canada *Identifying Genes Required for Saccharomyces cerevisiae Growth in Mucin*	**12.45 Frederik EISELE,** University of Gothenburg, Sweden *The Hsp90 Co-chaperone Sgt1 is a Novel Player in Cytosolic and ER Protein Quality Control and Aging*	**12.45 Celina BORGSTRÖM,** Lund University, Sweden *Identification of Modifications Procuring Growth on D-xylose in Recombinant Saccharomyces cerevisiae Strains Carrying the Weimberg Pathway*
13:00	Lunch, Speaker's corner, SGD Q&A, Coffee		
14:00	**DNA replication, mutation and repair** Chair: David Shore **Katrin PAESCHKE,** Universitätsklinikum Bonn, Germany 14:00-14:30 *Characterization of G4 function during post-replicative DNA repair* **Naama BARKAI,** Weizmann Institute of Science, Israel 14:30-15:00 *Two-step response to replication stress: a dual role of the replication checkpoint* **Maria Pia LONGHESE,** Università degli Studi di Milano, Bicocca, Italy 15:00-15:30 *Functions and regulation of the MRX complex at DNA double-strand breaks* **Camilla BJÖRKEGREN,** Karolinska Institute, Sweden 15:30-16:00 *DNA supercoiling – good or bad for chromosome stability?*		
16.00	Chair: Agnieszka Chacinska **Susan Gasser,** University of Basel, Switzerland *Tracking the global DNA damage response with single molecule imaging in yeast*		
17:00	Closing ceremony		

Social Events

XXIX INTERNATIONAL CONFERENCE ON YEAST GENETICS AND MOLECULAR BIOLOGY
ICYGMB 2019
Göteborg (Gothenburg) Sweden August 18–22, 2019

Welcome reception

Sunday, August 18 18:30-20:00 at the conference venue
(included in the registration fee)

Mingel food with drink
Music: *Blue Heaven Big Band (BBHB)* is a big band from Lerum (outside Gothenburg) who plays lovely dance music interspersed with concerts. The big band has been active for more than 30 years (!) and "still going strong". Over the years, the band has had the opportunity to play with artists such as Nils Landgren, Putte Wickman, Margareta Ewmark, Pierre Swärd and Magnus Lindgren, and many more

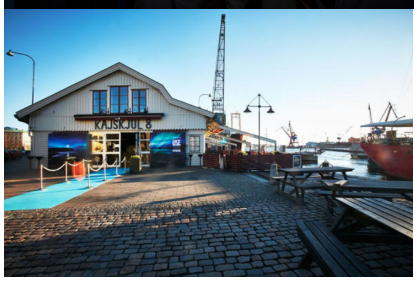
Poster session with music and bar

Tuesday, August 20 18:00-20:00 at the conference venue

Bar: Beer, wine and light snacks avalaible for purchase
Music: *Oh Amphibian* creates indie pop with a soulful voice and a rhythmic guitar based groove. Influenced by artists like Bon Iver and Paul Simon, Oh Amphibian takes the listener on an emotional journey amongst fragmented memories and elephants in the room.

Conference dinner

Wednesday, August 21 19:00-01:00 at Kajskjul 8 (separate fee)
Welcome: Surströmming tasting
Dinner: 3- course gala dinner
Music: *Gosskören* is a joy-maximization organization with a show-choir as a business concept. We believe in jokes and singing as a way to take Sweden out of the crisis. The choir took its first tentative attempt at the Stenhammarsalens showers after a spex performance in 1996. Gosskören is a show-choir that performs pop and rock'n roll songs in the acapella setting, they have performed in several places in Europe, USA and Asia.
Disco: DJ Homan Alipour



Poster Sessions

Monday August 19 even numbers
Tuesday August 20 odd numbers
Wednesday August 21 all

Ageing and disease models

46 Ruchika SACHDEV, ETH Zurich, Switzerland
Regulating the Dynamicity of Stress Induced Cytoplasmic Ribonucleoprotein Granules via Liquid-Liquid Phase Separation.

49 Teresa ZOLADEK, Institute of Biochemistry and Biophysics PAS, Poland
Yeast-Model-Based Study Identified Myosin- and Calcium-dependent Calmodulin Signalling as a Potential Target for Drug Intervention in Chorea-Acanthocytosis.

51 Pedro ORTEGA, University of Seville, Spain
A Novel Role For Histone Deacetylases In The Maintenance Of Genome Integrity

66 Alexander ALEXANDROV, Russian Academy of Sciences Moscow, Russia
Distinct patterns of cell death and division arrest at early replicative ages revealed by genome-wide screening.

78 Antonio MARSELLA, University of Milan – Bicocca, Italy
Rif2-mediated Regulation of MRX Activity at DNA Double-Strand Breaks

97 Erin BONNELL, Université de Sherbrooke, Canada
A Novel Regulator of Senescence.

103 Barry BOCHNER, Biolog Inc., United States
New Mitochondrial Function Assays Demonstrate Substantial Differences among Yeasts and Uncover Novel Metabolic Energy Pathways

106 Martin KUPIEC, Tel Aviv University, Israel
The Many Roles of Elg1 in the Maintenance of Genome Stability and Chromatin States

127 Alexander DELUNA, Centro de Investigación y de Estudios Avanzados de lPN, Mexico
Cellular mechanisms of longevity by dietary restriction revealed by large-scale competitive-aging screens

142 Humberto ITRIAGO, Lund University, Sweden
Either Rap1 or Cdc13 Can Protect Telomeric Single-stranded 3' Overhangs from Degradation in vitro.

143 Iñigo PRADA-LUENGO, University of Copenhagen, Denmark
Global Sequencing of Circular DNA Reveals Loss of Genetic Variation and the Role of Replication Origins for Maintenance of Circular DNA in Aging Yeast Cells

146 Théo ASPERT, IGBMC, France
Replicative Senescence In S. cerevisiae: A New Tool For A New Approach

148 Tiago OUTEIRO, University Medical Center Goettingen, Germany

Insights into the molecular underpinnings of Parkinson's disease: lessons from yeast

172 Xin CHEN, Chalmers University of Technology, Sweden
Physiological and Transcriptome Analyses of Amyloid-β Peptide-induced Cytotoxicity in Saccharomyces cerevisiae.

173 Asim SENGOR, ETH Zurich, Switzerland
Cytoplasmic age-dependent protein aggregates control DNA-circle accumulation in the aging mother cells in S.cerevisiae

175 Laura TROUSSICOT, University of Gothenburg, Sweden
The Peroxiredoxin-Hsp70 Interplay Slowing Down Yeast Replicative Aging Deciphered by NMR, a Molecular Microscope.

197 Linnea ÖSTERBERG, Chalmers University of Technology, Sweden
Oscillatory Behaviors in Snf1 Signaling Network.

200 Barbara SCHNITZER, Gothenburg University, Sweden
A Theoretical Approach to Understand the Role of the Retention Mechanism in the Rejuvenation Process

205 Katarína JURÍKOVÁ, Comenius University in Bratislava, Slovakia
Yeast Telomeric Protein Cdc13 Binds Secondary Structures on Telomeric DNA With Low Affinity.

208 Anton NIZHNIKOV, St. Petersburg State University, Russia
The Gln3 Transcriptional Regulator of the Nitrogen Catabolism Forms 'Conditional' Prion When Overproduced in Yeast Saccharomyces cerevisiae.

218 Maurizio David BARONI, University of Padua, Italy
Hydroxycitric Acid Can Antagonize Chronological Aging, Apoptosis and ROS-Induced Cell Death in Budding Yeast

221 Johannes BORGQVIST, University of Gothenburg, Sweden
The polarising world of Cdc42: the importance of geometry in cell division

223 Olga VYDZHAK, Institute of Molecular Biology Mainz/ Institute of Developmental Biology and Neurobiology Mainz, Germany
Acquisition of Genotoxin Resistance through Checkpoint Adaptation and Aneuploidy

226 Kanika SAXENA, University of Gothenburg, Sweden
Metabolic Buffering against Proteotoxic stress in Aging Cells.

228 Cecilia PICAZO, University of Gothenburg, Sweden
Role of the Peroxiredoxin Tsa1 in Protein Biosynthesis

239 Benjamin BARRE, IRCAN, France
Intragenic Repeat Expansions Control Yeast Chronological Aging.

244 Arthur FISCHBACH, University of Gothenburg, Sweden
An SGA-Based High-Content Microscopy Screen To Identify Genes That Control Protein Aggregate Handling During Aging.

245 Clara CORREIRA MELO, The Francis Crick Institute, United Kingdom
Metabolic Cooperation Modulates Eukaryotic Microbial Communities Ageing and Survival.

250 Veronica GAST, Chalmers University of Technology, Sweden
Exploring Saccharomyces Cerevisiae As A Production Platform For Biopharmaceuticals.

252 Sara N. MAVROVA, European Research Institute for the Biology of Ageing, Netherlands
Loss of Physicochemical Homeostasis in Yeast Replicative Ageing.

257 Hector GARCIA SEISDEDOS, Weizmann Institute of Science, Israel
Proteins Evolve on the Edge of Supramolecular Self-assembly.

280 Jurgita PAUKSTYTE, University of Helsinki, Finland
Age-Dependent Transitioning of Glutamate Synthase into Large-Scale Assemblies

288 Michelle LINDSTRÖM, University of Gothenburg, Sweden
Induction of Abnormal Protein Aggregation – A High-Content Imaging-based Screening of FUS Foci Formation in S. cerevisiae.

290 Carole LINSTER, University of Luxembourg, Luxembourg
Nicotinamide Nucleotide Damage and Repair in Yeast.

291 Navinder KUMAR, University of Gothenburg, Sweden
Hsp90 Immunophilin Homolog Cpr7 Is Required For [URE3] Prion Propagation In Yeast

303 Dieter SAMYN, Swedish University of Agricultural Sciences, Sweden
ILF and VRED Pathways Prevent Vacuole/Lysosome Rupture by Oxidative Stress.

307 Raffaele LOPREIATO, University of Padova, Italy
Nucleolin Rescues TDP-43 Cytotoxicity In A Yeast Model Of ALS Disorder.

308 Patrick NG, Stanford University, United States
Representing Transcriptional Heterogeneity and Inter-Strain Variation at the Saccharomyces Genome Database.

309 Per WIDLUND, University of Gothenburg, Sweden
Variation in Processing of Misfolding Proteins.

323 Frederik EISELE, University of Gothenburg, Sweden
The Hsp90 Co-chaperone Sgt1 is a Novel Player in Cytosolic and ER Protein Quality Control and Aging

324 Srishti CHAWLA, University of Gothenburg, Sweden
Ca2+/Calmodulin, Calcineurin and metacaspases : The 3-Cs of Protein Quality Control in Yeast.

338 Brandon HO, University of Toronto, Canada
Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae

356 Mahsa EBRAHIMI, Stockholm University, Sweden
Phosphate starvation promotes longevity via activation of autophagy and the MVB pathway in Saccharomyces cerevisiae

365 John- Patrick ALAO, University of East London, United Kingdom
Caffeine stabilizes fission yeast Wee1 in a Rad24- dependent manner but attenuates its expression under genotoxic conditions

371 Anna Maria EISELE-BÜRGER, University of Gothenburg, Sweden
Role of calmodulin in turning a metacaspase executioner into a protector

374 Rebecca ANDERSSON, University of Gothenburg, Sweden
The Different Roles of Cytosolic Hsp70s in Proteostasis and Lifespan Regulation
376 Wilhelm VAYSSE-ZINKHÖFER, Pasteur Institute, France
DNA double-strand break repair mechanisms and resection within a CAG/CTG microsatellites

384 Marjan ABBASI, Karolinska institutet, Sweden
Discovering Age-Specific Post-translational Modifications (PTMs) of Budding Yeast Centrosomes

Cell growth, division and morphogenesis

52 Lihi GERSHON, Tel Aviv University, Israel
A New Regulatory Pathway Responding to Hyper-Acetylation of Histone H3K56

62 Attila Csikász-Nagy, King's College London / Pázmány Péter Catholic University, Hungary
Measuring and Modelling Yeast Colony Growth

63 Bíborka PILLÉR, Pázmány Péter Catholic University, Hungary
Cellular Interactions in Yeast Colonies

68 Alexander LORENZ, University of Aberdeen, United Kingdom
Genome Stability, Karyotype Plasticity, DNA Damage Response, and Morphogenetic Switching in the Fungal Pathogen Candida auris

69 Robert WYSOCKI, University of Wroclaw, Poland
Mechanisms of antimony genotoxicity in budding yeast

73 Ireneusz LITWIN, University of Wroclaw, Poland
Snf2-like Protein Irc5 Facilitates Error-free DNA Damage Tolerance Pathway Through Cohesin

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Template-limited Transcription Coordinates Histone Homeostasis with DNA Content

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Start is not the Metabolic Commitment Point of the Yeast Cell Cycle

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Multiple Layers of Phospho-Regulation Coordinate Metabolism and the Cell Cycle

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The Telomeric DNA Terminal 5' end Sequence is Highly Regulated in the Budding Yeast Naumovozyma castellii

121 Igor KUKHTEVICH, Helmholtz Zentrum Muenchen, Germany
A Novel Method to Follow RNA Expression in Live Yeast Cells at the Single-Cell Level Over the Cell Cycle and Multiple Generations

131 Kurt M. Schmoller, Helmholtz Zentrum Muenchen, Germany
Coordination Of Protein Homeostasis With Cell-size In Budding Yeast

155 Severin EHRET, Humboldt-Universität zu Berlin, Germany
Cell Cycle Regulation at the Level of mRNA

158 Ines DE OYA, University of Seville - University Pablo de Olavide, Spain
A Novel Connection Between the Nuclear Pore Complex and Cell Cycle Regulation

160 Kate CAMPBELL, Chalmers University of Technology, Sweden
Metabolism is Tailored to Biosynthetic Needs During the Budding Yeast Cell Cycle

165 Adrianna SKONECZNA, Polish Academy of Science, Poland
Loss of Control at G1/S Phase Border of Cell Cycle in swi6Δ Cells of S. cerevisiae Leads to Genome Instability through Replication Stress-induced DSBs and Rad51-mediated Illegitimate Recombination

171 Min LU, Zhejiang University, China
Centromere repositioning causes inversion of meiosis and generates a reproductive barrier

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Role of the ScMep2 Ammonium Transport Protein in Filamentation Induction

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Yeast Telomeric Protein Cdc13 Binds Secondary Structures on Telomeric DNA With Low Affinity

215 Franziska WOJCIECH, University of Regensburg, Germany
eIF2 and Cdc123 in mRNA translation and cell cycle entry

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Acquisition of Genotoxin Resistance through Checkpoint Adaptation and Aneuploidy

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Identifying Genes Causing Genome Instability upon Overexpression in Saccharomyces cerevisiae

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An a priori Model for Yeast Cell Expansion During Vegetative Growth

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Quantifying Cellular Responses to Heat-Shock Using Transmission Electron Microscopy of High-Pressure Frozen Yeast Cells

277 Anika SEEL, Helmholtz Zentrum München, Germany
Coordination of Mitochondrial Homeostasis With Cell Size

279 Steven BOURNE, Aberystwyth University, United Kingdom
Real-Time Monitoring, Mathematical Modelling and Elucidation of the Morphological Transition of Candida tropicalis from Yeast to Pseudohyphae by Dielectric Spectroscopy

282 Sabrina HUELL, Ulm University, Germany
Nba1 is Involved in Cell Abscission During Cytokinesis

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A Mechanistically Detailed Model of the Cell Division Cycle in Saccharomyces cerevisiae

287 Nataliia KASHKO, Lomonosov Moscow State University, Russia
Cellular Control of Mitochondrial DNA Levels During Prolonged Cell-Cycle Arrest in Saccharomyces cerevisiae

293 Lara Bareis, Ulm University, Germany
Characterization of a Novel Member of the Yeast Polarisome With a Role in Actin Organization

296 Paul Cullen, SUNY-Buffalo, United States
The Role of Cdc42p Adaptors in Regulating the Filamentous Growth MAP Kinase Pathway

308 Patrick NG, Stanford University, United States
Representing Transcriptional Heterogeneity and Inter-Strain Variation at the Saccharomyces Genome Database

310 Narendra K Bairwa, Shri Mata Vaishno Devi University, India
Loss of ATG1 and YDR131C Together Results in Flocculation Behavior in S. cerevisiae.

318 Andreas CUNY, Technische Hochschule (ETH) Zürich, Switzerland
Cell Region Fingerprinting Improves Tracking Accuracy in Long-term Time-lapse Microscopy to Study the Inheritance of Dynamic Processes

339 Aleksandar VJESTICA, Univeristy of Lausanne, Switzerland
Coordinate regulation of the cell cycle and re-fertilization blocks ensures ploidy maintenance in fission yeast

351 Jan SKOTHEIM, Stanford University, United States
The biosynthetic basis of cell size control

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Phosphate starvation promotes longevity via activation of autophagy and the MVB pathway in Saccharomyces cerevisiae

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Uncovering Genes Involved in Cell-Cell Fusion During the Mating of Yeast

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Role of Eisosomal Proteins in Yeast Mating

387 Anson SHEK, Max Planck Institute of Molecular Physiology, Germany
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Differential Expression and Function of eIF5A Isoforms

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Optogenetic downregulation of protein levels with an ultrasensitive switch

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A New Regulatory Pathway Responding to Hyper-Acetylation of Histone H3K56

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Natural Variation and Heterogeneity in the Saccharomyces cerevisiae Lag Phase

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Study of Functional Modules Stability - Transcriptomic Analysis of Saccharomyces cerevisiae Genomes During the Laboratory Evolution

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Unusual DNA Binding Preferences of the Yeast AP-1-like Transcription Factor Yap8 Are Dictated by the N-terminal Ancillary Region Adjacent to the Basic Region

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Towards Establishing a Method to Measure Translation in Pichia pastoris

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Flocculation of Saccharomyces cerevisiae is Dependent on Activation of Slt2 and Rlm1 Regulated by the Cell Wall Integrity Pathway

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Ixr1 is an intrinsically disordered protein with prion-like characteristics

90 Artyom EGOROV, Moscow State University, Russia
The standard KanMX gene knockout cassette strongly affects the expression of neighboring genes at transcriptional and translational levels

93 Jennifer TATE, University of Tennessee Health Science Center, United States
Contrasting With Expectations, Sit4 and PP2A Dephosphorylate Transcription Activator Gln3 In Nitrogen-Excess, A Condition Where TorC1 Is Activated

94 Rosemary YU, Chalmers University of Technology, Sweden
Nitrogen Limitation Reveals Large Reserves in the Metabolic and Translational Capacities of Yeast

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Phosphate Homeostasis and Cell-to-cell Variation in PHO-gene Expression in Saccharomyces cerevisiae is Controlled through 14-3-3 Protein Bmh1, Spl2 and Non-coding RNA transcription

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Developing DhNik1 as a crowding responsive stress sensor

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Employing Artificial Promoters in Yeasts Using the Protein Expression Platform GeneEE

179 Regina SEPŠIOVÁ, Comenius University, Slovakia
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180 Filip ČERVENÁK, Comenius University, Slovakia
Runaway Evolution of Yeast Telomeric Repeats: In Search of the First Steps

183 Anamika SINGH, Indian Institute of Technology Delhi, India
The Mut+ Strain of Komagataella phaffii (Pichia pastoris) Expresses PAOX1 5 and 10 Times Faster Than Muts and Mut− Strains: Evidence That Formaldehyde or/and Formate Are True Inducers of AOX

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185 Derek WILKINSON, Charles University, Czechia
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The Well-Tempered Controller-846: A Precisely Titratable and Tight Transcriptional Controller in S. cerevisiae

194 Beatrice BERNARDI, Hochschule Geisenheim University, Germany
Short-flank PCR-based gene targeting in lager yeast based on RAD51 overexpression

205 Katarína JURÍKOVÁ, Comenius University, Slovakia
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207 Yury MALOVICHKO, All-Russia Research Institute for Agricultural Microbiology, Russia
The Divergent and Highly Specific Effects of the [SWI+] Prion Formation and SWI1 Deletion on the Transcriptome of the Yeast Cell

225 Maurizio MORMINO, Chalmers University of Technology, Sweden
Development of two Acetic or Lactic Acid Biosensors in Yeast

230 Iván Francisco CIKLIC, Chalmers University of Technology, Sweden
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231 Nozomu SAEKI, Okayama University, Japan
Systematic Identification of Genes whose Overexpression Positively Affect Fitness in Various Environments

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The SAGA/TREX-2 Subunit Sus1 Plays a Significant Role in the Adaptive Transcriptional Response Mediated Through the CWI Pathway in Saccharomyces Cerevisiae

240 Silvia SOTO DIAZ, Universite Libre de Bruxelles, Belgium
Characterization of the activity regulation of Mep-Amt-Rh ammonium transport proteins by TORC1-Npr1-Amu1/Par32 in Saccharomyces cerevisiae

243 Dimitra DIALYNAKI, University of Crete, Greece
The Anti-Cancer Drug Zeocin Affects Copper/Iron-Regulated Transcription and Causes Metabolic Reprogramming in S. cerevisiae

246 Albert SERRA-CARDONA, Columbia University, United States
Mechanisms Underlying the Distribution of Parental and Newly Synthesized H3-H4 Histones during DNA

Replication

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Exploring Saccharomyces Cerevisiae As A Production Platform For Biopharmaceuticals

261 Anna BABOUR, INSERM, France
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299 Amandine BONNET, Université de Paris, Institut de Recherche Saint-Louis, INSERM U944, CNRS UMR 7212, France
Multiple Functions Of The Nuclear Pore Complex In The Replication Cycle Of The Ty1 Retrotransposon

305 Antonia Maria ROMERO, University of Gothenburg, Sweden
The yeast AP-1 factor Yap8 couples arsenic-sensing to transcriptional regulation of target genes

306 Vaskar MUKHERJEE, Chalmers University of Technology, Sweden
Fine-tuning the stress response of Saccharomyces cerevisiae using CRISPR interference technology

308 Patrick NG, Stanford University, United States
Representing Transcriptional Heterogeneity and Inter-Strain Variation at the Saccharomyces Genome Database

311 Francisco SALINAS, Universidad Austral de Chile, Chile
Transcriptional Activity and Protein Levels for Horizontally Acquired Genes in Yeast Reveal Hallmarks of Adaptation to Low Nitrogen Content

312 Yvonne NYGÅRD, Chalmers University of Technology, Sweden
CRISPR interference technology for development of more tolerant industrial yeast strains

313 Natália BARBOSA, São Paulo State University, Brazil
eIF5A depletion impairs oxidative phosphorylation

314 Zdena PALKOVA, Charles University in Prague, Czechia
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320 Jason KUEHNER, Emmanuel College, United States
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325 Shira URIM, Technion, Israel Institute of Technology, Israel
"mRNA Imprinting": Regulation of Gene Expression by Co-transcriptionally Associating Proteins

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Simultaneous Quantification of mRNA and Protein in Single Cells Reveals Trans-acting Genetic Variation

336 Sviatlana SHASHKOVA, University of York, United Kingdom
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338 Brandon HO, University of Toronto, Canada
Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae

367 Neha JAIN, Max-Planck-Institute of Molecular Physiology, Germany
Recognition of H3 K14 Acetylation and H2B SUMOylation controls RSC Complex recruitment to Chromatin in vivo

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Heterologous gene function and drug discovery

47 Catarina BOURGARD, University of Gothenburg, Sweden
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50 Piotr SOCZEWKA, Institute of Biochemistry and Biophysics PAS, Poland
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Unusual DNA Binding Preferences of the Yeast AP-1-like Transcription Factor Yap8 Are Dictated by the N-terminal Ancillary Region Adjacent to the Basic Region

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208 Anton NIZHNIKOV, St. Petersburg State University, Russia
The Gln3 Transcriptional Regulator of the Nitrogen Catabolism Forms 'Conditional' Prion When Overproduced in Yeast Saccharomyces cerevisiae

209 Svenja BRAAM, University of Gothenburg, Sweden
Interactions of a Peroxiredoxin with the Proteasome in H2O2-induced Proteostasis.

214 Georgios KARRAS, University of Texas MD Anderson Cancer Center, United States
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217 Shan JIANG, University of Gothenburg, Sweden
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227 Lisa LARSSON, University of Gothenburg, Sweden
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244 Arthur FISCHBACH, Sahlgrenska Academy (University of Gothenburg), Sweden
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280 Jurgita PAUKSTYTE, University of Helsinki, Finland
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294 Kara SCHNEIDER, Sahlgrenska Academy (University of Gothenburg), Sweden
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295 Sansan HUA, University of Gothenburg, Sweden
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Proteome Re-Allocation Towards Amplified Translational Machinery as the Limiting Factor for Increased Growth Rate of Amino Acid Supplemented Saccharomyces cerevisiae.

322 Tony HAZBUN, Purdue University, United States
Elucidating the function of alpha-N-terminal protein methylation of Hsp31 and other N-terminal methyltransferase substrates in proteostasis and stress response

323 Frederik EISELE, Sahlgrenska Academy (University of Gothenburg), Sweden
The Hsp90 Co-chaperone Sgt1 is a Novel Player in Cytosolic and ER Protein Quality Control and Aging

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374 Rebecca ANDERSSON, Sahlgrenska Academy (University of Gothenburg), Sweden
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382 Marina Jecmenica, University of Natural Resources and Life Sciences, Austria
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384 Marjan ABBASI, Karolinska institutet, Sweden
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39 Lucia RAMOS-ALONSO, Instituto de Agroquímica y Tecnología de Alimentos IATA-CSIC, Spain
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46 Ruchika SACHDEV, ETH, Zurich (Institute of Biochemistry), Switzerland

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133 Ankita AWASTHI, Gautam buddha university, India
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135 Hanna ALALAM, University of Gothenburg, Sweden
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139 Kurt M. SCHMOLLER, Helmholtz Zentrum München, Germany
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155 Severin EHRET, Humboldt-Universität zu Berlin, Germany
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206 Alon APPLEBOIM, Hebrew University of Jerusalem, Israel
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Fine-tuning the stress response of Saccharomyces cerevisiae using CRISPR interference technology

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317 Karl-Dieter ENTIAN, Goethe University Frankfurt, Germany
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320 Jason KUEHNER, Emmanuel College, United States
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383 Gretchen EDWALDS-GILBERT, Claremont McKenna, Pitzer, Scripps Colleges, United States
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46 Ruchika SACHDEV, ETH Zurich, Institute of Biochemistry, Switzerland
Regulating the Dynamicity of Stress Induced Cytoplasmic Ribonucleoprotein Granules via Liquid-Liquid Phase Separation.

71 Ewa MACIASZCZYK-DZIUBINSKA, University of Wroclaw, Institute of Experimental Biology, Poland
Unusual DNA Binding Preferences of the Yeast AP-1-like Transcription Factor Yap8 Are Dictated by the N-terminal Ancillary Region Adjacent to the Basic Region.

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Turnover and Substrate Specificity of the Arsenite Transporter Acr3 from Budding Yeast.

81 Santhosh Kumar SARIKI, IISER BHOPAL, India
Flocculation of Saccharomyces cerevisiae is Dependent on Activation of Slt2 and Rlm1 Regulated by the Cell Wall Integrity Pathway.

82 Cristina VIEITEZ, EMBL, Germany
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83 Angel VIZOSO-VÁZQUEZ, CICA-Universidade da Coruña, Spain
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89 Dorota A. RZECHONEK, Wrocław University of Environmental and Life Sciences, Poland
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98 Marjolein CROOIJMANS, Leiden University, Institute of Biology, Netherlands
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99 Oliver KONZOCK, Chalmers University of Technology, Sweden
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137 Philipp SCHÖPPNER, Philipps-Universität Marburg, Germany
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145 Basile JACQUEL, IGBMC, France
NADPH deficiency reveals a tradeoff between adaptation and persistence under oxidative stress

149 Carmen WEBER, ETH Zurich, Institute of Biochemistry, Switzerland
Acute Glucose Starvation Induces Global Cytoplasmic Reorganization and Metabolic Reprogramming.

159 Agata TARCZYKOWSKA, Department of Chemistry and Molecular Biology, Gothenburg University, Sweden
A Graded Fission Yeast MAPK Response to H2O2 Requires Wis1 Thiol Inhibition and is Reversible by a Small Molecule.

161 Asha Densi K P, Indian Institute of Technology Bombay, India
Do Synonymous Mutations Have an Evolutionary Significance?

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Hydroxycitric Acid Can Strongly Antagonize Chronological Aging, Apoptosis and ROS-Induced Cell Death in Budding Yeast

220 Angela SELLERS MOYA, Universidad Politécnica de Madrid, Spain
Clotrimazole Deeply Alters MAPK Signalling in Saccharomyces cerevisiae and Other Yeasts.

224 Keisuke OBARA, Nagoya University, Japan
Development of a Biosensor for the Plasma Membrane Lipid Asymmetry.

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Development of two Acetic or Lactic Acid Biosensors in Yeast.

232 Mónica Pavón VERGÉS, Universidad Complutense de Madrid, Spain
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234 Per O. LJUNGDAHL, Stockholm University, Sweden
The ER Membrane Chaperone Shr3 Co-translationally Assists Biogenesis of Related Polytopic Membrane Protein Substrates.

238 Raphael LOLL-KRIPPLEBER, University of Toronto, Canada
Identifying Genes Causing Genome Instability upon Overexpression in Saccharomyces cerevisiae.

240 Silvia SOTO DIAZ, Universite Libre de Bruxelles, Belgium
Characterization of the activity regulation of Mep-Amt-Rh ammonium transport proteins by TORC1-Npr1-Amu1/Par32 in Saccharomyces cerevisiae

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The Hsp70 Homolog Ssb: a Novel Component of the TORC1/Sch9 Signaling Network

243 Dimitra DIALYNAKI, University of Crete, Greece
The Anti-Cancer Drug Zeocin Affects Copper/Iron-Regulated Transcription and Causes Metabolic Reprogramming in S. cerevisiae.

245 Clara CORREIA MELO, The Francis Crick Institute, United Kingdom
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249 Michael SCHWEIZER, Heriot-Watt University, United Kingdom
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Transcriptome Analysis Reveals the Moon-Lighting Role of Hsp31p in the Budding Yeast Cell Defense Against Fermentation-Related Stresses.

338 Brandon HO, University of Toronto, Canada
Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae

344 Norma Silvia SÁNCHEZ, UNAM, Mexico
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350 Allyson O'DONNELL, University of Pittsburgh, USA
Fluorogen Activating Proteins as a Powerful New Imaging Tool for Quantitative Protein Trafficking Studies in Yeast.

352 Martin SCHMIDT, University of Pittsburgh, USA
Spontaneous mutations that confer resistance to 2-deoxyglucose act through Hxk2 and Snf1 pathways to regulate gene expression and HXT endocytosis

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38 Danguole ZIOGIENE, Institute, Lithuania
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55 Jana HELSEN, VIB - KU Leuven, Belgium
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62 Attila CSIKÁSZ-NAGY, King's College London / Pázmány Péter Catholic University, Hungary
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63 Bíborka PILLÉR, Pázmány Péter Catholic University, Hungary
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66 Alexander ALEXANDROV, Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia
Distinct patterns of cell death and division arrest at early replicative ages revealed by genome-wide screening

71 Ewa MACIASZCZYK-DZIUBINSKA, University of Wroclaw, Institute of Experimental Biology, Poland
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Ancillary Region Adjacent to the Basic Region.

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Multiple Layers of Phospho-Regulation Coordinate Metabolism and the Cell Cycle.

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Constructing and Utilizing Transcriptional Regulatory Networks for Understanding and Redesigning Pathways in S. cerevisiae

144 Lina RIEGO-RUIZ, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), Mexico
Gln3 Links Nitrogen Assimilation With Fluconazole Resistance In Candida glabrata.

155 Severin EHRET, Humboldt-Universität zu Berlin, Germany
Cell Cycle Regulation at the Level of mRNA.

163 Brenda ANDREWS, University of Toronto, Canada
From Phenotypes to Pathways: Global Analysis of Subcellular Compartment Morphology using Systematic Genetics and Single Cell Imaging

171 Min LU, Life Sciences Institute, Zhejiang University, China
Centromere repositioning causes inversion of meiosis and generates a reproductive barrier

177 Jolanda van LEEUWEN, University of Lausanne, Switzerland
Systematic analysis of bypass suppressors of essential genes

184 Niek WELKENHUYSEN, Chalmers University of Technology, Sweden
Robustness of nutrient signalling is maintained by interconnectivity between signal transduction pathways

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202 Elie SALIBA, Molecular Physiology of the Cell Laboratory, ULB, IBMM, Gosselies, Belgium
Activation of TORC1 in Response to H+ Influx: Role of Pma1.

204 Duccio CAVALIERI, Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, Florence, Italy
Saccharomyces cerevisiae and Social Wasps as Models for the Evolution of Host Microbe Interactions.

207 Yury MALOVICHKO, All-Russia Research Institute for Agricultural Microbiology, Russia
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210 Roland TENGÖLICS, Biological Research Centre of the Hungarian Academy of Sciences, Hungary
Rapid Non-targeted Metabolite Profiling of Yeast

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Saturated Transposon Analysis in Yeast goes further.

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Production of soluble secreted mCherry in Pichia pastoris and its applications

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Uncovering Genes Involved in Cell-Cell Fusion During the Mating of Yeast

386 Sheila MAINYE, Max Planck Institute of Molecular Physiology, Germany
Role of Eisosomal Proteins in Yeast Mating

387 Anson SHEK, Max Planck Institute of Molecular Physiology, Germany
Identifying Novel Fusion Proteins in S. cerevisiae Mating Using Suppression and Sufficiency Criteria

1 Using yeast to map environment-dependent protein networks and functional human sequence variation

Frederick Roth

University of Toronto

Coupling the awesome power of yeast genetics with DNA sequencing technology has enabled experimentation at scales that are often limited only by our ability to design multiplexing strategies. First, I will cover the TileSeq strategy for measuring the functional effects of nearly all possible missense variants, and its potential to provide exhaustive variant effect maps for many human disease-associated proteins using yeast. Second, I will describe the Barcode Fusion Genetics (BFG) strategy for generating and tracking all combinations of two barcoded libraries, and its use for global mapping of protein interactions in yeast under multiple environmental conditions.

2 Chromosome Structure and TranscriptIon

Roger Kornberg

Stanford University

Three aspects of the nucleosome and of chromosome structure will be discussed: the role of the nucleosome; the +1 nucleosome of transcriptionally active genes; and the higher order structure of chromatin, specifically the basis for condensation in mitotic chromosomes. The principal role of the nucleosome is in transcriptional repression, modulated by the well known post translational modifications. The +1 nucleosome proves to be important for the initiation of transcription by RNA polymerase II. Condensation in mitosis is brought about by a “volume phase transition,” a first order phase transition as shown by the observation of hysteresis in condensation-decondensation curves.

3 Tracking the global DNA damage response with single molecule imaging in yeast

Susan Gasser

University of Basel

Nucleosomes are essential for proper chromatin organization and the maintenance of genome integrity. Histones are post-translationally modified and often evicted at sites of DNA breaks, mediated by chromatin remodelers, which shift, remodel and reassemble chromatin at sites of damage. Using quantitative imaging and proteomics methods we show that histone levels drop by 20-40% in response to extensive DNA damage, reflecting nucleosome eviction by the INO80 remodeler and degradation by the proteasome (Hauer et al., NSMB 2017). This occurs not only at sites of damage but genome-wide, in a manner dependent on the checkpoint kinase Mec1/ATR. Chromatin decompaction and increased fiber flexibility accompany histone degradation, and occur in the absence of damage when histone levels are reduced by other means. Changes in the physical properties of chromatin in response to DNA damage are monitored by single particle tracking. We now link these with changes in the "chromatome", that is, the set of proteins bound to chromatin before and after DNA damage is induced. Intriguingly, the S-phase loading of Cohesin not only reduces mobility due to sister-sister cohesion, but it also constrains the enhanced mobility that ensues from induction of a double-strand break (DSB). We examined whether whole chromosome anchoring through the centromere is altered in response to DNA damage, but we find no impact of DNA damage on kinetochore anchoring. Finally, we tested the role played by increased DNA accessibility due to histone loss in homology directed DSB repair. We score a striking enhancement in the rate of strand invasion into an ectopic site of homology, which is lost in the absence of Arp8, a crucial subunit of the INO80 remodeler. Reduced local mobility has less of an effect than the global increase in chromatin decompaction, arguing that histone depletion is a major driver in the homology search during recombination-mediated repair.

4 Crosstalk between transcription, splicing and chromatin

Jean Beggs

The University of Edinburgh

There is extensive evidence that in both metazoans and budding yeast the process of splicing occurs as soon as the intron is transcribed and before transcription termination, i.e. co-transcriptionally. As a result, RNA polymerase II elongation rate can influence splicing, and splicing can affect transcription elongation. More recently, links between splicing and chromatin modification have also become evident. We have investigated the interactions between these three important cellular processes in budding yeast. Using mutations in the large subunit of RNA polymerase II that alter transcription elongation rate, we obtained evidence that slow RNA polymerase II elongation increases both co-transcriptional splicing and splicing efficiency and that faster elongation reduces co-transcriptional splicing and splicing efficiency, indicating that splicing is more efficient when co-transcriptional. Moreover, we demonstrate that splicing accuracy is sensitive to transcription rate; altering RNA polymerase II elongation rate in either direction compromises splicing fidelity, especially for ribosomal protein gene transcripts.
We also analysed histone modifications following rapid degradation of individual splicing factors, finding that defects in specific stages of splicing differentially affect H3K4 and H3K36 tri-methylation. Based on our results, we propose that transcription and chromatin likely respond to signals from splicing fidelity checkpoints.

5 Nuclear mRNP Assembly and Export

Katja Sträßer, Philipp Keil, Alexander Wulf, Nitin Kachariya, Michael Sattler and Henning Urlaub

Justus Liebig University Giessen,Max Planck Institute for Biophysical Chemistry,Technical University Munich,Helmholtz Center Munich

An integral step of gene expression is the formation of an mRNP by assembly of nuclear RNA-binding proteins onto the mRNA and the subsequent export of the formed mRNP out of the nucleus. The function of the proteins involved in these processes have been largely analyzed by deletion or depletion of the whole protein or at least protein domains, which probably abrogates several functions of each protein at once. In order to determine specifically the RNA-binding function of proteins involved in nuclear mRNP assembly, we first determined the amino acids involved in RNA binding by RNPXL. We identified about 100 amino acids cross-linked to RNA in vivo in Npl3, Nab2, Tho1, Mex67-Mtr2, and the TREX complex. Second, we can now specifically elucidate the function of the RNA-binding activity of these proteins by mutation of the identified amino acids.

Here, we analyzed the RNA-binding function of Npl3, an SR-like protein with functions in transcription elongation, poly(A) tail formation, mRNP assembly, and nuclear mRNA export. The middle part of Npl3 consists of two RRM domains connected by an eight amino acid long flexible loop. In order to analyze the function of the RNA binding activity of Npl3, we changed amino acids that cross-linked to RNA. We generated two Npl3 mutants, one in the loop region, named npl3-loop, and one within RRM1, named npl3-RRM1, and elucidated the functional consequences of these mutations. Interestingly, npl3-loop leads to a nuclear mRNA export defect, while npl3-RRM1 does not. Furthermore, both mutants show distinct and specific changes in the composition of nuclear mRNPs. Thus, abrogation of mRNA-binding in different regions of Npl3 has different functional outcomes.

Taken together, we identify the in vivo RNA binding sites of nuclear mRNA binding proteins involved in mRNP assembly and nuclear mRNA export. In addition, we show that abrogation of RNA binding in different regions of the protein Npl3 has specific and surprisingly different functional consequences. Thus, our approach unraveled novel and unexpected insights into the process of nuclear mRNP assembly.

6 High throughput mutagenesis strategies to understand enzyme function and predict disease risk

Maitreya Dunham

University of Washington

Human genetics is currently overwhelmed by the huge amount of genetic variation being discovered by new sequencing efforts. One key limitation is the lack of corresponding functional annotation of these gene variants that would allow the field to link them to clinically actionable drug interactions. We are addressing this problem in yeast with an initial emphasis on important genes relevant for drug metabolism. In particular I will discuss our recent results with CYP2C9, which encodes an enzyme responsible for metabolizing many different drugs including warfarin, a widely prescribed oral anticoagulant with a narrow therapeutic window. Efforts to comprehensively characterize CYP2C9 and other pharmacogene variants have been hindered by the low-throughput nature of classic biochemical assays. Instead, we have developed a yeast-based activity assay that can test variants at high-throughput in a pooled manner. This assay, which uses activity-based protein profiling, is able to recapitulate the activity of known variants in both individual and pooled tests. Briefly, yeast cells expressing a single CYP2C9 variant are bound in an activity-dependent manner by a modified CYP2C9 inhibitor and are then labeled with a fluorophore for cell sorting and sequencing. Key improvements to the assay came from yeast strain background engineering. This included yeast humanization by adding other human metabolism enzymes, screening different yeast strain backgrounds, and making targeted strain background modifications. We have tested a library of thousands of single amino acid variants of CYP2C9 with our yeast-based assay. We will use this data to classify unknown variants and ultimately create a sequence-function map of CYP2C9 variants. Our approach will lead to advances in adverse drug response prevention by providing CYP2C9 clinical guidance for patients carrying both currently known and yet-to-be discovered alleles, and we hope it will template similar studies for additional human genes.

7 Metabolite exchange interactions between yeast cells, and how they create cellular heterogeneity

Markus Ralser

University of Cambridge

Cells excrete a large number of metabolites, as part of their intrinsic metabolic programme. This metabolite export enriches the extracellular space within communities. In this lecture, I show results that demonstrate the growth-relevant contribution of this extracellular metabolome, and how it enables cells to specialize in metabolism to generate single-cell heterogeneity.

8 Engineering Yeasts for Production of Glycolic Acid, a monomer for bioplastic PGA.

Merja Penttilä

VTT

An important metabolic engineering target with yeasts is the production of platform chemicals and monomers to be used for polymerisation into bioplastics. The most well-known example is the PLA monomer lactic acid (LA), which is already produced commercially with yeast. The glycolic acid (GA) polymer PGA has some superior material properties over PLA. However, engineering yeast to efficiently produce GA is much more challenging than LA production and requires considerations on for instance engineering the central carbon metabolism and glucose repression, as well the carbon source and the most suitable yeast species to be used for production. The engineering challenges and approaches will be presented.

9 Molecular mechanisms underlying stress granule formation, function and disease

Simon Alberti

Max Planck Institute for Cell Biology and Genetics

Stressed cells shut down translation, release mRNA from ribosomes, and form stress granules that may cause disease. The relationship between these activities remains enigmatic. We demonstrate that stress granules form by phase separation of the protein G3BP1. G3BP1 phase separation requires a phosphorylation-sensitive destabilization of intramolecular interactions among the disordered acidic region and the RNA-binding region, which triggers a transition from a compact to an extended state. The open state promotes multivalent interactions among G3BP1 and unfolded RNA that drive stress granule formation. Other proteins partition into G3BP1 condensates after their formation to promote stress granule maturation or induce a liquid-to-solid transition that may cause disease. By manipulating the phase behavior of G3BP1, we demonstrate that stress granules are not required for translation shutdown, but function as platforms to prevent RNA entanglement. In conclusion, we propose a molecular mechanism for how complex structures, such as stress granules, emerge through regulated density transitions coupling post-translational modifications, conformational rearrangements, and multivalent intermolecular interactions.

10 Stress responses to mitochondrial dysfunction

Agnieszka Chacinska

University of Warsaw

Mitochondria are multifunctional organelle, primarily involved in a fundamental biological process of respiration. The nuclear-encoded proteins make up for the large majority of proteins involved in the formation of mitochondria including the respiratory chain complexes. The efficient functioning of mitochondria depends on the proper transport, sorting and assembly of mitochondrial proteins that originate from the nuclear genome. Two main arms of the cellular response to protein import dysfunction include the inhibition of cytosolic translation and activation of the major protein degradation machinery, the proteasome. The

stimulation of the proteasome is driven by its more efficient assembly as a response to the amount of mistargeted proteins. The mechanism is beneficial for cells. Interestingly, activation of the proteasome could be uncoupled from translation effects. The synthesis of cellular proteins is regulated by the signals, which come directly from the dysfunctional mitochondria. To understand translational inhibition, a site-specific redox proteomic analysis to delineate the yeast redoxome was performed. Increased levels of intracellular reactive

oxygen species (ROS) caused by the mitochondria serve as a signal to attenuate global protein synthesis. Mapping of redox-active thiols in proteins revealed ROS-sensitive sites in several components of the translation apparatus. Thus, the increased levels of intracellular ROS caused by dysfunctional mitochondria serve as a signal to attenuate global protein synthesis. Thus, several mechanisms exist that link the status of mitochondria with regulation of the cellular protein homeostasis.

11 Identifying new players involved in spatial sequestration of protein aggregates

Thomas Nyström

University of Gothenburg

Spatial sorting to discrete quality control sites in the cell is a process harnessing the toxicity of aberrant proteins. We have found that the yeast t-snare phosphoprotein, syntaxin5 (Sed5), acts as a key factor in mitigating proteotoxicity and the spatial deposition and clearance of IPOD (insoluble protein deposit) inclusions associates with the disaggregase Hsp104. Sed5 phosphorylation promotes dynamic movement of COPII-associated Hsp104 and boosts disaggregation by favoring anterograde ER-to-Golgi trafficking towards the vacuole and mitochondria. Many inclusions become associated with mitochondria in a HOPS/vCLAMP-dependent manner and co-localize with Vps39 (HOPS/vCLAMP) and Vps13; proteins providing contacts between vacuole and mitochondria. Aggregate association with mitochondria and the Vps39 and Vps13 proteins are required for efficient Sed5-dependent clearance of aggregates.

12 Single cell analysis of entry into replicative senescence: do budding yeast really age?

Gilles Charvin

IGBMC Strasbourg

In budding yeast, a mother cell undergoes a limited number of divisions before it enters senescence and dies. Although many molecular and cellular events have been shown to correlate with replicative age, whether and how they are temporally and causally linked during the transition to senescence remains elusive. In this study, we used microfluidics and single-cell imaging to monitor in real-time one of these key events, the nucleolar accumulation of extrachromosomal rDNA circles (ERCs), throughout the lifespan of yeast cells and to assess its temporal and causal relationship to the onset of senescence. Our single cell data unambiguously reveal that ERCs accumulate rapidly with exponential kinetics well before senescence onset, and this is concomitant with marked upregulation of the rDNA transcription machinery but not of downstream components of ribosome biogenesis. This breakdown in nucleolar coordination is rapidly followed by loss of nuclear homeostasis, including dysregulation of nuclear transport, which immediately precedes the onset of cell cycle slowdown, suggesting a series of causally related events. Finally, our data reveal that asymmetrical division of the nucleolus and nucleus, rather than unequal partitioning of ERCs alone, drives the recovery of physiological function in daughters of aged mother cells. Using a simple computational analysis, we show that our data support a quantitative model in which a probabilistic age-independent event (ERC excision) triggers a temporally ordered cascade of nucleolar and nuclear events that ultimately lead to cell death. Hence, our study suggests that replicative aging in budding yeast is an emergent property, arising from age-independent cellular processes.

13 Holistic control by molecular networks in proliferation and aging

Marti Aldea

CSIC

Loss of proteostasis and cellular senescence are key hallmarks of cell aging, but whether they are subject to direct cause-effect relationships is not known. The most upstream activator of cell cycle entry in budding yeast is Cln3, a G1 cyclin that critically depends on molecular chaperones to accumulate in the nucleus in late G1. On the other hand, chaperones are massively involved in key growth processes, and we had previously proposed that coordination between proliferation and growth relies on the competition for chaperones within one of the largest molecular networks in cells, i.e. the chaperone-client network. Thus, we wanted to test whether this competition scenario would also operate in aged cells, where proteostasis decline may seriously compromise chaperone function. We have found that most yeast cells arrest in G1 before death with low nuclear levels of cyclin Cln3. As expected, chaperone availability is strongly reduced in aged cells, and the G1 arrest coincides with the massive aggregation of a metastable chaperone-activity reporter. A mathematical model integrating autocatalytic protein aggregation and central components of Start network recapitulates empirical observations. As key predictions, G1-cyclin overexpression increases lifespan in a chaperone-dependent manner, and lifespan reduction by enforced protein aggregation is greatly alleviated by increased expression of specific chaperones or cyclin Cln3. Overall, our data indicate the crucial role of chaperone malfunction in setting lifespan in yeast cells, and configure a molecular pathway whereby proteostasis breakdown acts as a direct effector of cell senescence.

14 Cell fate control by an alternative transcriptome

Folkert van Werven

The Francis Crick Institute

Long noncoding RNAs (lncRNA) and alternative mRNA isoforms make up a large fraction of the transcriptome and play key functions in cell-fate programming. These alternative transcripts can regulate local gene expression in cis through transcription-coupled chromatin alterations. How transcription of some cis-acting RNAs leads to activation of gene expression, while others inhibit and repress gene expression remains poorly understood. We investigated in S. cerevisiae the functions of the alternative transcriptome by 1) studying an lncRNA upstream in the promoter of the master regulator for entry into meiosis, and 2) cataloguing the expression of all transcript isoforms throughout the different transitions of the yeast meiotic program. We report the surprising finding that distinct levels of lncRNA transcription regulates opposing chromatin and transcription states to ensure that only diploids, and not haploids, enter meiosis and form gametes. Additionally, by studying distinct cell fate transitions of the yeast meiotic program, we identified several new features of regulating local gene expression by transcription of alternative RNAs. I will discuss these features and their implications for understanding gene regulation and cell fate control. We propose that transcription of lncRNAs and mRNAs isoforms plays an important role in regulating local gene expression, and changes in their transcription levels can confer distinct regulatory and cell fate outcomes.

15 Control of cell identity by nuclear pore acetylation

Manuel Mendoza

IGBMC Strasbourg

The acquisition of cellular identity is coupled to changes in the nuclear periphery and nuclear pore complexes (NPCs). Whether and how these changes influence cell identity remain unclear. We have uncovered a mechanism that regulates NPC acetylation to determine key aspects of cell identity after asymmetric division in budding yeast. The lysine deacetylase Hos3 associates specifically with daughter cell NPCs during mitosis to delay cell cycle entry (Start). Hos3-dependent deacetylation of nuclear basket and central channel nucleoporins establishes daughter-cell-specific nuclear accumulation of the transcriptional repressor Whi5 during anaphase and perinuclear silencing of the G1/S cyclin gene CLN2 in the following G1 phase. Hos3-dependent coordination of both events restrains Start in daughter, but not in mother, cells. I will summarise these findings and present new data suggesting that deacetylation of nuclear pores plays a broader role in nuclear transport and gene positioning in daughter cells. In particular, Hos3 modulates the perinuclear localisation of factors involved in mRNA synthesis and export, and the interaction of inducible genes with the nuclear periphery during their activation, suggesting a key role for NPC acetylation in the control of gene expression during cell differentiation. We propose that nucleoporin deacetylation is a cell fate determinant in asymmetrically dividing budding yeast, opening the possibility that similar mechanisms might contribute to establishment of distinct cellular states in yeast and animal cells.

16 A Novel and Robust Molecular Design Synchronizing Transcriptional with Cell Cycle Oscillators

Matteo Barberis

University of Surrey

Some biological networks exhibit oscillatory behavior of their components with a frequency that is of functional importance to convert stimuli to time-dependent responses. The eukaryotic cell cycle is such a case, being governed by waves of cyclin-dependent kinase (cyclin/Cdk) activities that rise and fall at a specific frequency and thereby guarantee its timely occurrence. Disruption of cyclin/Cdk oscillations could result in dysfunction through reduced cell division. Therefore, molecular designs that exhibit cyclin/Cdk oscillations are inherently crucial for a timely cell cycle progression. Although details about transcription of cyclins, the regulatory subunits of Cdk, are available, a lack of understanding exists about network motifs responsible for the precise timing of cyclin/Cdk oscillations.

Recently, we have identified a transcriptional cascade that regulates the relative timing of waves of mitotic (Clb) cyclin expression in budding yeast. This involves the Forkhead (Fkh) transcription factors, conserved among eukaryotes. Here we investigate the network motifs responsible for timely cyclin/Cdk oscillations, with the aim to unravel the molecular design that interlocks Clb waves through Fkh-mediated signaling.

An integrated computational and experimental framework is presented. A kinetic model of a minimal cyclin/Cdk network is verigied against in vivo quantitative data of Clb dynamics. Robustness analyses are then performed by testing a number of possible network motifs for their ability (i) to resemble Clb oscillations and (ii) to generate sustained oscillations in the form of limit cycles. A novel regulatory design, coined as Multiplex Phase Interlocker (MPI), is unravelled, with a linear, Fkh-mediated cascade among Clb cyclins and Clb/Cdk1-mediated positive feedback loops being pivotal for synchronizing Clb/Cdk1 oscillations. Furthermore, our model predicts a definite Fkh activation pattern underlying this design, with a progressive Clb/Cdk1-mediated Fkh phosphorylation. Experimental validation confirms the computational prediction, highlighting the Clb/Cdk–Fkh axis being pivotal for timely cell cycle dynamics.

Altogether, our integrative approach pinpoints how robustness of the cell cycle control is realized, by revealing a novel and robust principle of design that ensures a timely interlock of transcriptional with cyclin/Cdk oscillations.

17 Creating a Functional Single Chromosome Yeast

Zhongjun Qin

Shanghai Institute of Plant Physiology and Ecology

Eukaryotic genomes are generally organized in multiple chromosomes. Here we have created a functional single chromosome yeast from a Saccharomyces cerevisae haploid cell containing sixteen linear chromosomes through successive chromosome end-to-end fusions and centromere deletions. The fusion of sixteen native linear chromosomes into a single chromosome result in dramatic changes in the global chromosomal three-dimensional structure due to the loss of all centromere-, most of the telomere-associated inter-chromosomal interactions and 67.4% of intra-chromosomal interactions. However, the single-chromosome and wild-type yeast cells have nearly identical transcriptome and similar phenome profiles. The giant single chromosome can support cell life, but displays less fitness in several aspects such as growth across environments and competitiveness, gametes production and viability. This synthetic biology study paves a new path for exploring the eukaryote evolution with respect to chromosome structure and function.

18 Solar Panels On Yeast: Inorganic Biohybrids For Enhanced Photochemical Production

Neel Joshi

Harvard University

The intersection between synthetic biology and materials science is an underexplored area with great potential to positively affect our daily lives, with applications ranging from manufacturing to medicine. Inorganic-biological hybrid systems have potential to be sustainable, efficient, and versatile chemical synthesis platforms by integrating the light-harvesting properties of semiconductors with the synthetic potential of biological cells. We have developed a modular bioinorganic hybrid platform that consists of highly efficient light-harvesting indium phosphide nanoparticles and genetically engineered Saccharomyces cerevisiae: a workhorse microorganism in biomanufacturing. The InP particles are adhered to the yeast surface using a versatile polyphenol-based chemistry. The yeast harvests photo-generated electrons from the illuminated nanoparticles and uses them for the cytosolic regeneration of redox cofactors. This enables the decoupling of biosynthesis and cofactor regeneration, facilitating a carbon- and energy-efficient production of the metabolite shikimic acid – a common precursor for several drugs and fine chemicals. We observed higher per-cell production of shikimic acid for biohybrids compared to their unmodified counterparts, in a light-dependent manner, and a global metabolic shift toward shikimic acid from other fermentative byproducts, like glycerol and ethanol. Our work provides a platform for the rational design of biohybrids for efficient biomanufacturing processes with higher complexity and functionality. The biohybrid polyphenol-based fabrication scheme can easily be adapted to virtually any combination of particle and cell type, greatly expanding the range of accessible systems and facilitating the exploration of structure-function relationships. Additionally, the vast toolbox of available genetically modified yeast strains will enable further probing of electron transport mechanisms and applicability to a wide variety of biochemical targets.

19 Two Different Strategies to Engineer Yeast for Production of Aromatic Compounds

Eckhard Boles

Goethe-University

We will present two alternative strategies for the synthesis of valuable aromatic compounds with the yeast Saccharomyces cerevisiae. For this, we either used the aromatic amino acid pathway to produce mandelic or 4-hydroxymandelic acid (1,2), and we used polyketide synthases to produce m-cresol (3). Mandelic and 4-hydroxymandelic acid are valuable specialty chemicals used as precursors for flavors as well as for cosmetic and pharmaceutical purposes. m-cresol (3-methylphenol) is utilized as disinfectant, act as an antioxidant scavenging reactive oxygen species, and is an important platform compound for synthesis of several chemicals with high market value like menthol.

For the production of mandelic or 4-hydroxymandelic acid (more than 1 g/L) we expressed and engineered heterologous hydroxymandelate synthases and optimized the flux into and through the phenylalanine or tyrosine branches, respectively, of the aromatic amino acid pathway. For the production of m-cresol (more than 0.5 g/L) we expressed the polyketide synthase methylsalicylic acid synthase together with a methylsalicyl acid decarboxylase. Limiting factors for the production of the different compounds were determined to be either the competition between mandelic and 4-hydroxymandelic acid production, or the toxicity of m-cresol.

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(2) Metab Eng Commun. 2018;7:e00079. doi: 10.1016/j.mec.2018.e00079.
(3) Hitschler J and Boles E, 2019. De novo production of aromatic m-cresol in Saccharomyces cerevisiae mediated by heterologous polyketide synthases combined with a 6-methylsalicylic acid decarboxylase. Metab Eng Commun. (accepted, in press).

20 Synthetic biology of yeast

Jens Nielsen

Chalmers University of Technology

Synthetic Biology relies on the Design-Build-Test cycle. This cycle includes technologies like mathematical modeling of metabolism, genome editing and advanced tools for phenotypic characterization. In recent years there have been advances in several of these technologies, which has enabled faster development of metabolically engineered strains that can be used for production of fuels and chemicals.

The yeast Saccharomyces cerevisiae is widely used for production of fuels, chemicals, pharmaceuticals and materials. Through metabolic engineering of this yeast a number of novel industrial processes have been developed over the last 10 years. Besides its wide industrial use, S. cerevisiae also serves as an eukaryal model organism, and many systems biology tools have therefore been developed for this organism. These tools can be used for detailed phenotypic characterization as well as for metabolic design.

In this lecture it will be demonstrated how the Design-Build-Test cycle has allowed for development of yeast cell factories for production of a range of different fuels and chemicals. Some examples of different technologies will be presented together with examples of metabolic designs, in particular for development of platform strains that can be used for production of a fatty acid derived products, e.g. fatty alcohols and alkanes. It will be argued that with advancement in genome-editing technologies and novel methods for rapid phenotypic screening, advancement in the field is hampered by our design abilities, i.e. to predict genotype-phenotype connections. For this genome-scale metabolic models is a strong technology, and in the presentation recent advancements in the integration of mathematical modeling with multi-omics analysis for cell factory design will be presented.

23 Functions and Regulation of the MRX Complex at DNA Double-Strand Breaks

Maria Pia Longhese

Università degli Studi di Milano - Bicocca

DNA double-strand breaks (DSBs) are highly cytotoxic lesions that must be repaired to ensure genomic stability and avoid cell death. Eukaryotic cells possess two main pathways for repairing DSBs: nonhomologous end-joining (NHEJ) and homologous recombination (HR). While NHEJ requires little or no processing of DNA ends, HR is initiated by nucleolytic degradation of the 5' terminated strands at both DNA ends by a concerted action of nucleases in a process termed DNA end resection. The evolutionarily conserved Mre11-Rad50-Xrs2/NBS1 complex (MRX in budding yeast, MRN in humans) is among the first protein complexes that are recruited at DSBs. MRX plays an important role in controlling end resection and in maintaining the DSB ends tethered to each other for their repair. Furthermore, it is implicated in recruitment and activation of the protein kinase Tel1 (ATM in mammals), which plays an important role in DSB signalling. Structural studies have shown that these diverse MRX/MRN functions are regulated by the ATP binding and hydrolysis activity of Rad50 that induce conformational changes of both Rad50 and Mre11. I will present and discuss hypomorphic and hypermorphic point mutations within the MRX subunits that has advanced our understanding of the functions and regulations of this multifunctional enzyme in DSB metabolism and Tel1/ATM activation.

24 Characterization of G4 function during post-replicative DNA repair

Katrin Paeschke

Universitätsklinikum Bonn

G4 structures are implicated to influence, positively and negatively, a variety of biological processes in the cell. Although many helicases have been demonstrated to unfold G4 efficiently only a handful of other G4-binding/stabilizing proteins have been identified so far. Using a yeast one-hybrid analysis we have identified Zuo1 as a novel G4-binding protein. In vitro binding analysis confirmed that Zuo1 binds specifically to G4 structures.

To transfer the in vitro data to the cellular function we detected genome-wide by chromatin immunoprecipitation (ChIPseq) all regions to which Zuo1 binds. These analyses confirmed that Zuo1 binds to G4 motifs in vivo. In subsequent vitality, genetic and molecular analyses we further characterized the biological relevance of this interaction. These data revealed that Zuo1 recognizes folded G4 structures near DNA damage sites. This interaction has a positive effect on the recruitment of post-replicative DNA repair factors. The here presented data shows for the first time how a protein- G4 interaction results in a positive effect on DNA repair events.

25 DNA supercoiling – good or bad for chromosome stability?

Camilla Björkegren

Karolinska Institutet

Cohesin, condensin and the Smc5/6 complex (Smc5/6) belong to the family of SMC protein complexes, known to control chromosome replication, segregation, repair, and transcription. Cohesin is most well-known for its essential role in sister chromatid cohesion, condensin in chromosome condensation, and Smc5/6 in DNA repair and recombination. Chromosome segregation also fails in unchallenged cells lacking Smc5/6, but the reason for this remains mostly unknown.

We have shown that *Saccharomyces cerevisiae* Smc5/6 is largely absent from chromosomes in the G1 cell cycle phase, but colocalizes with cohesin in intergenic regions between genes that are transcribed in a convergently oriented manner after replication. Interestingly, Smc5/6 specifically associates to cohesin sites that are clustered around centromeres, and Smc5/6 enrichment at these sites correlates with the centromere-telomere distance, depends on sister chromatid cohesion, and decreases when a long chromosome is divided into two smaller variants. Additional Smc5/6 binding sites, also these overlapping with cohesin, appears along chromosome arms after inhibition of topoisomerase 2 (Top2) during S-phase. Altogether this suggest that Smc5/6 chromosomal association is controlled by DNA topology. This include DNA supercoiling, i.e. over- or under-twisting of the DNA helix by the replication- and transcription machineries, and sister chromatid entanglement. Our new investigations now suggest that Smc5/6 binding is dynamic and requires ongoing expression of convergently oriented genes. These results, and their implications for the understanding of Smc5/6 function and supercoiling of the replicated genome, will be discussed.

26 Mapping drivers of phenotypic change at single nucleotide resolution

Daniel Jarosz

Stanford University

Understanding the origins of diversity among individuals and species is a central challenge of genetics. Yet most genome-wide association studies cannot distinguish causal variants from linked passenger mutations. We have combined theory and experiment to overcome this challenge, pushing inbred crossing to its practical limit in Saccharomyces cerevisiae to improve the resolution of linkage analysis from kilobases to nucleotides. This 'super-resolution' approach has allowed us to map thousands of variants affecting growth in dozens of environments. All types of polymorphism (missense, synonymous, and cis-regulatory variants) collectively give rise to phenotypic diversity, providing mechanistic insight into the basis of evolutionary divergence. Our resolution has allowed us to establish that most traits are extremely complex – driven by multiple coding and non-coding variants alike – and revealed that multiple closely linked driver mutations frequently act on the same trait. Finally, we have used this platform to investigate the mechanisms responsible for complex heritability in natural genotype-to-phenotype maps. This approach complements traditional deletion and overexpression screening paradigms, opening new frontiers in quantitative genetics and providing valuable lessons that can be extended to other organisms.

27 Functional profiling of inter-genic and intronic non coding RNAs

Daniela Delneri

The University of Manchester

The Saccharomyces cerevisiae genome has undergone extensive intron loss during its evolutionary history, and the few remaining may be retained because of their impact on function in specific environmental conditions. We explored the possibility that new functional ncRNAs are embedded within intronic sequences and are responsible to intron retention in yeast. We employed de novo RNA structure prediction tools to screen intronic sequences in 37 fungi, and we identified and validated 19 novel intronic RNAs via RT-PCR. We deleted the novel intronic RNA structure within the GLC7 intron and showed that this region, rather than the intron itself, is responsible for the cell's ability to respond to salt stress. RNA-seq analysis confirmed that introns in ribosomal protein genes are more highly expressed when they contain predicted RNA structures. Overall, these data support the notion that some introns may have been maintained in the genome because they harbor important RNA structures.
Our lab has also been involved in the construction of a barcoded ncRNA deletion collection in S. cerevisiae (ca. 450 mutants; Ref 2), and we are currently creating phenotypic and genetic interaction maps of ncRNAs in S. cerevisiae by exploiting (i) fitness analysis of double KO mutants generated using Synthetic Genetic Array (SGA) and (ii) screening the double KO library in stress conditions. Data on large-scale functional analysis (i.e. bar-seq and transcriptome data) and synthetic genetic interactions for the ncRNA collection will be presented: to date 27 ncRNAs have been used as query strains for SGA and several gene interactions have been discovered showing either loss or gain in fitness, such as for example ΔCUT084-ΔSUT083 or ΔSNR17-ΔCUT150.

28 Antifungal drug responses of cells and populations

Judith Berman

Tel Aviv University

Treatment of fungal infections in animals, or fungal infestations of crops, requires an understanding of how much of an antifungal agent needs to be applied, how often to use it and what types of responses to expect. In general, clinical studies have focused on the minimum inhibitory concentration: the concentration of drug at which 50% or more of the cells exposed to the drug do not grow. Above this concentration, cells are said to be susceptible. However, many patients infected with susceptible isolates suffer persistent or recurrent infections despite being treated with drug concentrations above the MIC for the infecting organisms. Phenomena known as tolerance, trailing growth, hetero-resistance or persistence have been noted and are due to subpopulations of cells that grow slowly in the presence of the drug. These subpopulations, together with the selective pressure of drug treatment acting upon them, have the potential to drive the evolution of new mechanisms for survival of the pathogen. We have been studying tolerance and hetero-resistance in Candida albicans and Candida glabrata, respectively. This talk will highlight the growth dynamics, physiological responses and evolutionary responses of subpopulations that survive and grow, albeit slowly, in the presence of severe antifungal stress. One of the mechanisms of survival in drug involves changes in chromosome stoichiometry, usually via aneuploidy and its effects on the expression of specific genes.

29 Multiple reinventions of mating-type switching during budding yeast evolution

Kenneth Wolfe

University College Dublin

Mating-type switching originated at least 11 times independently during the evolution of budding yeasts, on different branches of the phylogenetic tree. We reached this conclusion by inferring mating compatibility systems from the genome sequences of 332 yeast species whose phylogeny was recently published. Based on the presence of MATa and/or MATα genes, and the presence or absence of DNA repeats flanking these genes, we were able to classify each species as either heterothallic (self-infertile) or homothallic (self-fertile), and to divide the homothallic species into primary homothallics (where any cell can mate with any other cell) and secondary homothallics (where cells can switch mating types). The 3-locus mating type switching system (MAT/HML/HMR) as seen in S. cerevisiae has a single evolutionary origin, but we identified 10 other clades that each independently evolved flip/flop switching systems of the type found in Ogataea polymorpha. We identified 31 evolutionary transitions from heterothallism to homothallism, but only 3 transitions in the opposite direction, indicating that self-fertility has a strong evolutionary advantage. The 3-locus system predated the origin of HO endonuclease, which is derived from a mobile genetic element.

30 Adaptation to Osmostress by the HOG1 SAPK

Francesc Posas

Pompeu Fabra University

Exposure of cells to osmostress results in the activation of the Hog1/p38 family of stress-activated protein kinases (SAPKs). Activation of these highly conserved MAP kinases 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, in yeast there is a major downregulation of gene expression that is bypassed specifically in stress-responsive genes by the action of the Hog1 SAPK which acts in multiple stepts of mRNA biogenesis. In addition to regulate transcription, SAPKs control cell cycle progression. For instance, Hog1 modulates the G1/S transition by targeting core components of the cell cycle machinery such as CDK inhibitors as well as by regulating cell cycle gene expression. In addition, a novel checkpoint in S phase controlled by SAPKs is critical to coordinate transcription and replication allowing for full stress-responsive transcription during S phase without affecting DNA integrity. All together highlights the relevance of this signaling pathway in the control of several aspects of the cell physiology to maximize cell survival in the presence of stress.

31 Compartmentalized Synthesis of Triacylglycerol Regulates Nuclear Membrane Homeostasis During Stress.

Symeon Sinniossoglou

University of Cambridge

Cells dynamically adjust organelle organization in response to growth and environmental cues. This requires the regulation of synthesis of phospholipids, the building blocks of organelle membranes. Fatty acids (FAs) are essential components of phospholipids, but also of triacyglycerols (TGs) that enable energy storage in lipid droplets. Phospholipid and TG synthesis mainly take place at the endoplasmic reticulum membrane. How cells control the allocation of FAs between phospholipids, for membrane growth, and TG, for energy storage, remains unclear. We will present evidence of a lipid remodelling pathway operating at a subdomain of the inner nuclear membrane to regulate nuclear structure. We find that this pathway results in the synthesis of TG in response to cell-cycle and nutrient starvation signals and its compartmentalization is critical for nuclear integrity. Finally, we will discuss evidence supporting the presence of a mechanism that directs nuclear TG to the cytoplasmic side of the nuclear membrane.

32 Cellular decision-making in changing environments

Peter Swain

The University of Edinburgh

Cells have been selected for change, but yet are often studied in static environments. Here I will use two examples of signal transduction in budding yeast to demonstrate that dynamic inputs and monitoring outputs over time can give insight into how cells make decisions. First, I will show that ramping up an input can reveal how a trade-off in speed versus accuracy is mitigated in the response of yeast to hyperosmotic stress. Second, I will focus on how environmental identity is encoded intracellularly by studying the dynamics of nuclear translocation for 10 transcription factors in different types and levels of stress. Together the results show that network structures that might appear redundant play distinctive roles when viewed dynamically.

33 Networks for Calcineurin, the Ca2+/calmodulin activated phosphatase in yeast and humans reveal evolution of signaling

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Protein phosphatases, such as calcineurin, the conserved Ca2+/calmodulin-regulated phosphatase, are essential for cell signaling, but have proved difficult to study at the systems-level. We leveraged insights into calcineurin substrate recognition to systematically map its signaling network in yeast and humans. In yeast, calcineurin signaling promotes survival during environmental stress and downregulates pheromone signaling by regulating diverse processes from gene expression to membrane-trafficking. In humans, calcineurin has roles in the immune, cardiovascular and nervous systems, and is inhibited by the immunosuppressant drugs, FK506 and Cyclosporin A. Recognition of substrates and regulators by calcineurin occurs through a conserved mechanism: binding to Short Linear Motifs (SLiMs), termed PxIxIT and LxVP, which occur in intrinsically disordered domains, have low affinity for calcineurin and are degenerate in sequence.

To define the calcineurin signaling network in S. cerevisiae, we combined phosphoproteomics and bioinformatics, identifying proteins that contain a PxIxIT motif and have calcineurin-dependent phosphosites. We identified >30 substrates to reveal new functions. Furthermore, analyses of closely related yeasts showed that many proteins only recently acquired a calcineurin-binding SLiM (PxIxIT), indicating rapid evolution of the network.

We recently defined the human calcineurin signaling network in humans using unbiased SLiM-discovery methods: In vitro proteome-wide detection of CN-binding peptides, in situ SLiM-dependent proximity labeling, and in silico modeling of motif determinants uncovered unanticipated CN interactors. Unexpectedly, CN shows SLiM-dependent proximity nuclear pore complex (NPC) proteins, where Ca2+ signaling is largely uncharacterized. CN promotes accumulation of a nuclear reporter in HeLa cells, and dephosphorylates human (Nup153, Nup50) and yeast NPC proteins (Nup1, Nup2, Nup60), suggesting that regulation of the NPC by calcineurin is conserved.

Our studies provide critical new insights into Ca2+ and calcineurin signaling, and establish novel experimental and computational approaches to elucidate any SLiM-based signaling network. They also reveal a critical role for SLiMs, which evolve rapidly due to their location within regions of intrinsic disorder, in rewiring signaling networks.

347 Intracellular phosphate reception and signaling: A novel homeostatic system with roles for an "orphan" organelle?

Andreas Mayer

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Cells face a phosphate challenge. Growth requires a minimal concentration of this limiting resource because intracellular phosphate (Pi) is a compound of nucleic acids and modifies most cellular proteins. At the same time, cytosolic Pi may not rise much, because elevated cytosolic Pi can stall metabolism. It reduces the free energy that nucleotide triphosphate hydrolysis can provide to drive energetically unfavorable reactions.

Our group tries to elucidate how cells strike this critical balance. We characterize a novel pathway for intracellular phosphate reception and signaling (INPHORS), which may regulate cytosolic Pi homeostasis by coordinating multiple SPX-domain-containing proteins for import and export of Pi, and for Pi storage in acidocalcisomes. Acidocalcisomes are conserved but very poorly understood organelles. We propose that their role in buffering cytosolic Pi concentration is one reason for their evolutionary conservation.

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34 OLEFINE: OLEAginous Yeast Platforms For FINE Chemicals.

Leonie Wenning, Carina Holkenbrink, Baojian Ding, Hong-Lei Wang, Marie Inger Dam, Kanchana R Kildegaard, Christina Sinkwitz, Bettina Lorántfy, Christer Löfstedt and Irina Borodina

BioPhero ApS,Lund University

The ban of three neonicotinoids used as insecticides was settled in April 2018 by the European Union. At the same time, agricultural productivity needs to rise to provide sufficient nutritious food for a growing population. Moreover, a recent study predicts an increase in global yield losses of rice, maize and wheat by 10-25% per degree of global mean surface warming due to increased population growth and metabolic rates of insects. All these facts illustrate a pressing need for alternatives to conventional pesticides.

Pheromones, which are naturally produced by different insect species, represent a health- and environment-friendly alternative to insecticides. They can be used for mating disruption, a process in which small amounts of insect sex pheromones are released in the field to prevent the males from finding the insect females. This way females do not get fertilized and can not lay eggs that develop into larvae eating the plants. Pheromones are attractive, because they are biodegradable, species-specific compounds, which neither harm beneficial species nor humans. Nevertheless, pheromones are still not widely used, mainly because of the high costs of chemical synthesis. Therefore, the goal of the EU-funded project OLEFINE (www.olefine.eu) is the sustainable and cost-efficient production of insect pheromones by fermentation in modified oleaginous yeasts.

35 Unique Genetic Basis of the Distinct Antibiotic Potency of High Acetic Acid Production in the Probiotic Yeast Saccharomyces cerevisiae var. boulardii.

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VIB & KU Leuven

The yeast Saccharomyces boulardii has been used world-wide as a popular, commercial probiotic, but the basis of its probiotic action remains obscure. It is considered conspecific with budding yeast S. cerevisiae, which is generally used in classical food, bioethanol production and other industrial applications. They have an almost identical genome sequence, suggesting that they belong to the same species, which renders the genetic basis of the probiotic potency of S. boulardii even more unclear. We now show that S. boulardii produces at 37°C unusually high levels of acetic acid, which are strongly inhibitory to bacterial growth in agar-well diffusion assays and could be vital for its unique application as probiotic among yeasts. We were able to cross this trait through a tetraploid S. boulardii/S. cerevisiae hybrid strain into a diploid sporulation-competent hybrid strain and a mating-competent haploid segregant. Using pooled-segregant whole-genome sequence analysis with this haploid hybrid strain and an S. cerevisiae reference strain, we succeeded in mapping the underlying QTLs, and identified mutant alleles of SDH1 and WHI2 as the causative alleles. Both genes contain a SNP unique to S. boulardii (sdh1F317Y and whi2S287*), not present in the many sequenced S. cerevisiae strains and fully responsible for acetic acid production. S. boulardii strains show different levels of acetic acid production, depending on the copy number of the whi2287* allele as well as the sugar level in the medium. Our results offer the first molecular explanation as to why S. boulardii could exert probiotic action as opposed to S. cerevisiae. They reveal for the first time the molecular-genetic basis of a probiotic action-related trait in S. boulardii and show that antibacterial potency of a probiotic microorganism can be due to strain-specific mutations within the same species. We suggest that acquirement of antibacterial activity through medium acidification offered a selective advantage to S. boulardii in its ecological niche and resulted in its specific selection and application as probiotic.

36 Exploring genome architecture and stability in the absence of any single gene.

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While identification and analysis of genes required for maintaining genomic stability have traditionally relied on indirect assays and on the study of individual gene-deletions, whole-genome sequencing technologies now enable, in principle, the direct observation of genome instability globally and at scale. Here, we describe tools to extract information on copy-number variation of tandem and interspersed repetitive DNA elements, chromosomes and mitochondrial DNA from whole-genome sequencing data. With these, we have surveyed the Saccharomyces cerevisiae gene knockout collection to characterize genomic instability caused by the absence of any single non-essential gene (http://sgv.gurdon.cam.ac.uk). Analysis of this dataset reveals genes affecting maintenance of various genomic elements, highlights cross- talks between nuclear and mitochondrial genome stability, and shows how strains have adapted to life in the absence of a non-essential gene.

37 Approaching Single-cell “Epigenetic” Memory using Microfluidics.

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Mechanistically how transcriptional and “epigenetic” states are inherited through cellular divisions is currently only poorly understood. This inheritance of epigenetic states offers an important memory mechanism, for example, in response to environment or during cellular differentiation. However to define the heritability of epigenetic states within a population of cells is difficult due to cell heterogeneity in the level and stability of the underlying mechanisms. Therefore novel single-cell approaches to observe these processes in live cells, combined with high-throughput screening to identify factors that are involved are important for understanding how chromatin states and epigenetic modulators mediate “epigenetic” memory.

We have implemented a state-of-the-art microfluidics approach to address dynamics of Saccharomyces cerevisiae Gal1 transcriptional memory and in particular epigenetic inheritance at the level of individual yeast cells across multiple generations. We further applied a high-throughput microfluidics approach in combination with genome-wide screening to identify factors that are potentially implicated in the memory of transcriptional and/or chromatin signatures. Currently we are studying the mechanistic contribution of several factors identified in the screen to transcriptional and epigenetic memory with classic chromatin techniques and probing the specific effects on the inheritability of these states via single cell-tracking microfluidics. Our cell-tracking setup has allowed us to specifically observe maintenance of memory within individual cells and more intriguingly the inheritance of memory in their naïve progeny. We have further delved into the factors regulating noise of Gal1 gene induction and the specific contribution to noise in the inheritance of transcriptional memory.

We will present our novel workflow and the latest single-cell results about the inheritance of “epigenetic” states and the mechanisms mediating this inheritance.

38 Identification of Kluyveromyces Lactis Dolichol Kinase Mutations that Enhances Secretion of Heterologous Proteins.

Danguole Ziogiene, Albertas Timinskas and Alma Gedvilaite

Institute

Kluyveromyces lactis is biotechnologically significant yeast which has already been exploited as a host for the production of heterologous proteins due to its secretory performance. We have isolated mutant MD2/1-9 responsible for a super-secretion phenotype in K. lactis using a Bacillus amyloliquefaciens alpha-amylase as a marker for secretion. The genomes of both parental and mutated strains were sequenced using the next-generation sequencing. After analysis of sequences ORF5776 was selected for further studies. The protein sequence encoded by ORF5776 was 63% identical to dolichol kinase (DK) of K. marxianus and 43% identical to DK of S. cerevisiae, which is encoded by SEC59 gene. Based on this similarity, we concluded that the mutated protein in K. lactis is likely DK, which catalyzes CTP-dependent phosphorylation of dolichol, and named the gene KlSEC59. In S. cerevisiae sec59-1 conditional mutant incubated at a restrictive temperature produces incompletely N-glycosylated proteins and displays reduced secretion of vacuolar carboxypeptidase Y (CPY), invertase, and alpha-factor. The DK gene sequence in the K. lactis MD2/1-9 mutant strain had one G/A substitution, which resulted in a G405S amino acid substitution compared to the sequence of the parental MD2/1 strain. To confirm that the G405S mutation is responsible for the super-secretion phenotype in MD2/1-9 strain, we reverted it by applying CRISPR-Cas9 technology and a new introduced S405 mutation in parental MD2/1 strain. This reversion of the S405 mutation to G restored secretion of α-amylase in MD2/1-9 yeast cells to the level of secretion in parental MD2/1 strain and, vice versa, the mutation of G405 to S in parental MD2/1 strain enhanced secretion of α-amylase to the level of MD2/1-9 strain. Further we examined the KlSEC59 gene mutation G405S effects on the glycosylation of CPY and found that parental MD2/1 strain before mutagenesis already had some CPY glycosylation deficiency. As in Genbank databases there are two versions of the KlSEC59 gene encoding DK with three mismatches we showed that mutation S419I in DK of MD2/1 strain restored its CPY glycosylation deficiency. Introduction of G405S and I419S mutations into wild type strain CBS2369 confirmed that despite lower DK activity which results in some CPY and invertase glycosylation deficiency but has fewer effects on cell wall integrity, double mutations are responsible for enhanced secretion phenotype in K. lactis. (Grant number S-MIP-17-88).

39 The RNA-binding Protein Cth2 Regulates Respiration During Iron Deficiency

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Iron is a key micronutrient and cofactor for all eukaryotic organisms. Despite being essential for biological processes including respiration, DNA replication and reparation, and ribosome biogenesis, iron deficiency is a very common nutritional disorder due to its extremely low solubility at physiological pH. In consequence, organisms have developed regulatory mechanisms to adjust iron-dependent metabolism to cofactor bioavailability. In the model organism Saccharomyces cerevisiae, low iron activates two transcription factors, Aft1 and Aft2, that induce the expression of 25-30 genes, known as the iron regulon, to enhance the acquisition and recycling of extracellular and intracellular iron, respectively. In addition, important global metabolic changes take place via the iron regulon member Cth2.

Cth2 is an mRNA-binding protein that specifically interacts through its tandem zinc-fingers, conserved in plants and mammals, with multiple mRNAs to promote degradation and repression of translation. Most Cth2 mRNA targets encode for proteins that participate in highly iron-consuming pathways. The tricarboxylic acid cycle and the electron transport chain (ETC) include more than 20 putative Cth2 mRNA targets, suggesting a probable role of Cth2 in repressing respiration during iron deficiency. Here, we explore how Cth2 expression influences the mRNA and protein levels of respiratory genes, and its effect on oxygen consumption and specific enzymatic activities. We show that a hyperactive AFT1 allele decreases oxygen utilization and growth capacity in respiratory carbon sources through CTH2 activation. Moreover, overexpression of CTH2 limits oxygen consumption, even in iron-sufficient conditions. We have also observed specific reductions in the protein and activity levels of ETC complexes when CTH2 is overexpressed. All these results highlight the importance of Cth2 in regulating the respiratory capacity of yeast cells, especially during iron limitation.

40 A comparative study of the vacuolar CCC1/VIT1 iron transporter in the Saccharomyces genus

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Iron excess can be harmful for cells since it catalyzes the generation of reactive oxygen species (ROS) through Fenton reactions, causing cellular damage at the level of lipids, proteins and nucleic acids. In the budding yeast Saccharomyces cerevisiae, iron accumulation is regulated at the level of acquisition and storage since there is no mechanism for iron excretion. When iron is present at high levels, the yeast transcriptional factor Yap5 activates the expression of a few genes involved in iron detoxification and storage. The most representative Yap5-target gene is CCC1 (Ca2+ sensitive Cross-Complement 1), a vacuolar iron/manganese transporter homologous to VIT1 (Vacuolar Iron Transporter) in plants, that introduces the metal into the vacuole. A recent work has elucidated the crystal structure of Eucalyptus grandis Vit1 protein and has identified multiple motifs required for its metal transport mechanism (Kato et al., 2019). Using this structural information and many genome sequences, we have explored the diversity of Ccc1/Vit1 protein domains among living organisms. Particularly, Ccc1 proteins from the genus Saccharomyces mostly differ in their cytosolic amino-terminal region. We have explored the accumulation and resistance of all described Saccharomyces species and their important available populations to an excess concentration of iron.

41 Overproduction of Bioactive Molecules in Yeast and Evaluation of Their Effect Against Different Stresses.

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Microorganisms are responsible of the production of different bioactive molecules that are present in food and they have beneficial properties for health even in low quantities. Hydroxytyrosol is a natural polyphenol antioxidant that exists in olive oil in a minor amount, but it has been described as the most bioactive component in it. It is known that the synthesis of certain bioactive molecules, such as hydroxytyrosol or melatonin, occurs during alcoholic fermentation. Since Saccharomyces cerevisiae is the main responsible for this process, the direct relationship with the production of the compounds has recently been studied. However, the conditions and the genes involved in the production are still under investigation.

In this work, we aim to obtain different yeast strains that heterologously overproduce hydroxytyrosol, serotonin, N-acetyl serotonin and melatonin to evaluate the physiological effect of these overproductions on yeast cells growing under different stresses. We assess yeast growth performance under oxidative, osmotic and temperature pressures in monoculture and in competence experiments during fermentation. To date, hydroxytyrosol overproduction in yeast has still never been conducted and, together with overproduction of other bioactive compounds, it is an interesting way to optimize fermentation processes and ultimately improve bioactive properties in food products.

42 Differential Expression and Function of eIF5A Isoforms

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eIF5A is an essential protein in all eukaryotes involved in cell proliferation and animal development. At the molecular level, eIF5A acts as a translation elongation factor which binds to ribosomes to facilitate the translation of certain peptide motifs such as stretches of consecutive prolines. eIF5A is the only eukaryotic protein containing hypusine, an aminoacid that seems to be essential for eIF5A function. Most eukaryotes contain two highly homologous isoforms of eIF5A. In humans, EIF5A-1 gene is constitutively expressed in most cells types, while EIF5A-2 is only expressed in testis and brain. However, both genes are found over-expressed in many tumor types and have been linked to cancer metastasis. Understanding the differential regulation of the eIF5A isoforms and whether they have the same molecular functions in cells are important questions, but our current knowledge on these aspects is still very limited. Using Saccharomyces cerevisiae, we investigated the regulation of the two paralogous genes, TIF51A and TIF51B, encoding 91% identical isoforms of eIF5A, which are functionally redundant to the human proteins. Under normal growth conditions Tif51A is the most abundant isoform in yeast, but its expression was further increased when cells were grown with non-fermentable carbon sources, such as glycerol or ethanol. Conversely, the change of glucose to glycerol/ethanol still reduced the low levels of TIF51B mRNA. Accordingly, temperature sensitive mutants of Tif51A were able to grow in glucose but not in glycerol/ethanol under semi-permissive temperature. On the other hand, TIF51B mRNA is known to increase under anaerobiosis, when no respiration occurs. Additionally, we observed that exposure to iron deficiency, a condition where respiration is compromised, caused a decrease in TIF51A levels and a moderate increase in TIF51B levels. Our results show that under iron deficiency TIF51A mRNA levels are at least partially regulated by the post-transcriptional RNA-binding factor Cth2. In addition, LIA1, encoding an iron-containing enzyme that catalyzes the last step of hypusination, is also a Cth2 target and is downregulated under iron deficiency, which opens the possibility of a differential importance of hypusination for the function of each isoform. Our data suggest that Tif51A is necessary for the mitochondrial function and Tif51B would be the functional isoform under non-respiring conditions.

43 Engineering the availability of key precursor malonyl-CoA in Saccharomyces cerevisiae

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Malonyl-CoA is a precursor of a variety of compounds such as polyketides and flavonoids. In Saccharomyces cerevisiae, as a building block of fatty acid production, malonyl-CoA concentration is tightly regulated and therefore maintained at a very low level, limiting the production of malonyl-CoA-derived chemicals.

In this work, the availability of malonyl-CoA were optimized through transcription regulation and the mutation of acetyl-CoA carboxylase (Acc1p). We designed a synthetic malonyl-CoA biosensor and used it to screen phosphorylation site mutations of Acc1p. A combination of three site mutations in Acc1p, was found to increase malonyl-CoA availability. In addition, we also manipulated the phospholipid synthesis transcriptional regulators to control the malonyl-CoA levels and increase the downstream product. Through manipulating different regulators including Ino2p, Ino4p, Opi1p, and a series of synthetic Ino2p variants, combining with studying the inositol and choline effect, the engineered strain achieved a 9-fold increase of the titer of malonyl-CoA-derived product 3-hydroxypropionic acid, which is among the highest improvement relative to previously reported strategies. Our study provides valuable strategies to regulate malonyl-CoA availability and will contribute to the production of other highly valued malonyl-CoA-derived chemicals.

44 Study of Skin and Nail Candida species as a Normal flora based on Age groups in Healthy Persons in Tehran-Iran (2016)

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The skin is the body's largest organ that hosts heterogeneous inhabitants. Until now, the diversity of the cutaneous microbiome was mainly investigated for bacteria and there is a little information about the skin fungal flora. Also among skin fungal flora, Candida is found as a main member which it's distribution is affected by sex, age, climate. In this study, differences in Candida community structure associated with 9 different skin sites of 238 healthy people during 10 months from July to March 2016, are described. These subjects were divided by age into 4 groups: infants, children, adults and geriatrics. The collected samples were examined by culture on Sabouraud Chloramphenicol Agar and CHROM-agar Candida. For precise identification of species ITS1-5. 8S-ITS2 rDNA regions was sequenced where needed. The frequency of Candida species was significantly different between age groups. The most Candida isolations were related to the elderly age group and the fewest in the infants. C.parapsilosis virtually, was the predominant isolated species in all age groups. This study showed no statistically significant effect of the subject's sex on Candida population resident on human skin surface.

45 Optogenetic downregulation of protein levels with an ultrasensitive switch

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Optogenetic control of protein activity is a versatile technique to gain control over cellular processes, e.g. for biomedical and biotechnological applications. Among other techniques, the regulation of protein abundance by controlling either transcription or protein stability found common use as this controls the activity of any type of target protein. Here, we report modules of an improved variant of the photo-sensitive degron module and a light-sensitive transcription factor, which we compared to doxycycline-dependent transcriptional control. Given their modularity the combined control of synthesis and stability of a given target protein resulted in the synergistic down regulation of its abundance by light. This combined module exhibits very high switching ratios, profound downregulation of protein abundance at low light-fluxes as well as fast protein depletion kinetics. Overall, this synergistic optogenetic multistep control (SOMCo) module is easy to implement and results in a regulation of protein abundance superior to each individual component.

46 Regulating the Dynamicity of Stress Induced Cytoplasmic Ribonucleoprotein Granules via Liquid-Liquid Phase Separation.

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Regulation of gene expression is essential for cells to respond to different environmental cues and developmental changes. Key regulatory events occur post-transcriptionally and, for example, in response to certain stress conditions, cytoplasmic mRNAs localize to membraneless organelles such as processing bodies (PBs) and stress granules (SGs).

PBs and SGs are dynamic, phase-separated cellular compartments involved in degradation and/or storage of mRNAs and assemble via a variety of multivalent but weak RNA-protein, protein–protein and RNA-RNA interactions; a phenomenon called liquid–liquid phase separation (LLPS). Yet, how cells regulate assembly and disassembly of these membraneless organelles remains poorly characterized.

Our lab recently identified the DEAD-box ATPase Dhh1 as a critical regulator of PB dynamics. Mutations in Dhh1 that prevent ATP hydrolysis, or that affect the interaction between Dhh1 and Not1, the central scaffold of the CCR4-NOT complex and an activator of the Dhh1 ATPase, prevent PB disassembly in vivo. Interestingly, this process can be recapitulated in vitro, since recombinant Dhh1 and RNA, in the presence of ATP, phase-separate into liquid droplets that rapidly dissolve upon addition of Not1 (Mugler et al., 2016).

Here, we demonstrate that Pat1 a scaffolding protein involved in mRNA decay and translational repression antagonizes Not1 and promotes PB assembly via Dhh1. Pat1 binding to Dhh1 induces oligomerization and promotes PB assembly in vivo. Intriguingly, this can be recapitulated in vitro, since recombinant Dhh1 and RNA, in the presence of ATP, phase-separate into liquid droplets, a process, which is enhanced by the addition of recombinant Pat1 (Sachdev et al., 2019). Our results provide novel insight into the mechanisms of how cells control PB assembly and disassembly in different growth conditions.

Intriguingly, mRNP granules can also undergo maturation and solidification, and the aberrant formation of irreversible mRNP granules has been implicated in disease development. We will present results that shed light on this poorly understood process and its functional consequences in adaptation to stress and impact on cellular health and aging.

47 The Neglected Vivax Malaria Case: coupling Plasmodium spp. and Humanized Yeast Surrogate Genetics with In Silico Modelling for Target-based Drug Discovery

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Plasmodium vivax is a neglected but significant parasite causing human malaria, whose biology remains poorly understood. The geographical spread of P. vivax transmission, the limited therapeutic options, and the emerging resistance to antimalarials currently in use, seriously affect the likelihood of worldwide elimination of malaria in the near future. Hence, exploration of Plasmodium spp. molecular targets for new drug discovery and development is a priority and our main research goal. Thirteen P. vivax genes have been selected in silico according to three main criteria: a) gene orthology between P. vivax, H. sapiens for drug selectivity determination, S. cerevisiae, which acts as a surrogate for expressing parasitic targets, P. falciparum and P. berghei for downstream in vitro and in vivo validation; b) gene essential in yeast for functional complementation in constructed strains; and c) no paralogs in Plasmodium, yeast or human to avoid functional redundancy. We have genetically engineered S. cerevisiae strains to express six heterologous P. vivax and corresponding human molecular targets. Using protein modeling and virtual ligand screening, prospective compounds against three different Plasmodium spp. targets have been identified from synthetic libraries. For one specific P. vivax target, an essential eukaryotic ubiquitous enzyme catalyzing the covalent attachment of fatty acid to the N-terminal glycine of susceptible proteins, chemical screens to find antiplasmodial agents are ongoing. We already identified 4 out of 65 tested compounds from the Pathogen Box library from Medicines for Malaria Venture (MMV), that selectively inhibit the yeast strain expressing the P. vivax molecular target. Activity tests for these compounds against P. falciparum in erythrocyte culture show moderate parasite inhibition, with non-impairing cytotoxicity rates. Tests against a panel of P. falciparum strains resistant to clinically used drugs will further elucidate the potential of these compounds. Promising compounds with antiparasitic activity in vitro, in vivo and ex vivo will be chemically redesigned for improved selectivity. This Swedish-Brazilian-Cambodian funded joint research project uses high-throughput target-based methodology coupled with in silico target modeling for Plasmodium spp. candidate drug screening as a powerful approach to find new high efficacy antiparasitic lead molecules.

48 Systems analysis and engineering of an oleaginous red yeast

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Lipids produced by oleaginous yeasts have been recognized as important renewable resources for biofuels and oleochemicals. The red yeast Rhodosporidium toruloides is superb for production of neutral lipids under various conditions. To understand the molecular bases of its oleaginicity, we sequenced the genome of R. toruloides and performed trans-omic analysis. Our results demonstrated that TAG accumulation under nitrogen-limited conditions is tightly connected with cellular processes related to lipogenesis, nitrogenous compounds recycling, macromolecules metabolism and autophagy. Under phosphorus-limited conditions, cells activate degradation of phosphorus-containing components including RNA and nucleic acids such as adenosine monophosphate, leading to reduced cell proliferation and enhanced lipid biosynthesis. Genetic tools have been developed over the years and are applied to confirm major trans-omic observations. Also, advanced strains have been designed for production of various products derived from the fatty acid biosynthesis pathway or isoprenoid biosynthesis pathway.

49 Yeast-Model-Based Study Identified Myosin- and Calcium-dependent Calmodulin Signalling as a Potential Target for Drug Intervention in Chorea-Acanthocytosis.

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Chorea-acanthocytosis (ChAc) is a rare neurodegenerative disease associated with mutations in the human VPS13A gene. The mechanism of ChAc pathogenesis is unclear. A simple yeast model was used to investigate the function of Vps13. Vps13, like hVps13A, is involved in vesicular protein transport, actin cytoskeleton organisation and phospholipid metabolism. A new phenotype of the vps13Δ mutant, SDS-hypersensitivity, was used to screen a yeast genomic library for multicopy suppressors. A fragment of the MYO3 gene, encoding the N-terminal part of myosin, a protein involved in the actin cytoskeleton and in endocytosis, was isolated. Myo3-N protein contains a motor head domain and a linker. The linker contains IQ motifs that mediate the binding of calmodulin, a negative regulator. Amino acid substitutions that disrupt the interaction of Myo3-N with calmodulin resulted in the loss of vps13Δ suppression. Production of Myo3-N downregulated the activity of calcineurin, a protein phosphatase regulated by calmodulin, and alleviated some defects in early endocytosis events. Importantly, EGTA, which sequesters calcium and thus downregulates calmodulin and calcineurin, was a potent suppressor of vps13□. We propose that Myo3-N acts by sequestering calmodulin, downregulating calcineurin and activation of Myo3, which is involved in endocytosis and in Osh2/3 dependent endoplasmic reticulum-plasma membrane contact sites. These results show that defects associated with vps13Δ could be overcome and point to a functional connection between Vps13 and calcium signalling as a possible target for chemical intervention in ChAc. Yeast ChAc models may uncover the underlying pathological mechanisms, may also serve as a platform for drug testing. This study was financed by the National Science Centre Poland (UMO-2015/19/B/NZ3/01515) and published by Soczewka et al., Disease Models and Mechanisms (2019) 12 (http://dmm.biologists.org/content/12/1/dmm036830.long).

50 Using Yeast Chorea--acanthocytosis Model for Drug Screening.

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Chorea-acanthocytosis (ChAc) is a fatal rare genetic neurodegenerative disease caused by mutations in hVPS13A gene, one of four VPS13 genes in human. Mutations in hVPS13B, hVPS13C and hVPS13D 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 vps13 mutations. In yeast, there is one VPS13 gene and it is most homologous to hVPS13A. The deletion of VPS13 gene (vps13Δ) in yeast impairs many functions such as intracellular trafficking, actin cytoskeleton organization and maintenance of mitochondrial DNA. Recently, we discovered that vps13Δ cells are hypersensitive to SDS. This novel and simple growth phenotype was useful for our genetic screen for multicopy suppressors of vps13Δ mutation. Now, we used SDS hypersensitivity phenotype for isolating chemical suppressors of vps13Δ. We performed a drug screen using Prestwick Chemical Library, a collection of1280 chemical compounds, most of which is accepted for use in human. Based on the screen results and literature, we selected 7 substances, which suppress SDS hypersensitivity phenotype of vps13Δ, for further research. We analysed impact of these substances on other phenotypes of vps13Δ mutant, such as canavanine hypersensitivity, impaired Sna3 transport, mitochondrial DNA escape and impaired actin cytoskeleton organisation. Our work aims on selecting drugs with the highest therapeutic potential for studies on ChAc with use of cell lines and higher model organisms. Our findings may contribute in future to discovery of an effective therapy for ChAc and other diseases associated with VPS13 genes. This study is funded by the National Science Centre Poland (UMO 2015/19/B/NZ3/01515).

51 A Novel Role For Histone Deacetylases In The Maintenance Of Genome Integrity

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DNA replication failures is one of the most common causes of genome instability. When the replisome encounters single strand breaks, which are a common type of DNA lesion, it can give rise to double-strand breaks (DSBs). DSBs that arise during replication are preferentially repaired by sister chromatid recombination (SCR). Failures using the sister chromatid as a template to repair the damage lead to genome instability, a hallmark of cancer cells. We developed new tools to study SCR based on a mutated flipase (FLP), which induces mostly DNA nicks on either the leading or lagging DNA strand, allowing us to the study the repair of DSBs originated during replication. Through a screening of chromatin modifier and remodeler mutants, we identified different histone deacetylases that act as specific regulators of SCR without affecting the repair with other donor sequences. Instead, the loss of these histone deacetylases lead to an increase in chromosome loss, rearrangements and hyper-recombination between ectopic DNA sequences. An extended recombination analysis of double mutant combinations of these mutants with HR and SCR affected mutants reveals a possible mechanism by which histone acetylation regulates SCR during replication. The data presented here allow us to propose a novel role for histones deacetylases favoring the repair of replication-born DSBs by SCR avoiding genome instability

52 A New Regulatory Pathway Responding to Hyper-Acetylation of Histone H3K56.

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Acetylation of histone H3 on lysine 56 (H3K56Ac) is a modification that marks newly synthesized histones in the model system Saccharomyces cerevisiae. H3K56Ac was also found to be enriched at sites of DNA damage. Deacetylating this residue is equally as important as its acetylation. Two redundant proteins of the sirtuin family, Hst3p and Hst4p, keep the acetylation levels low outside the S-phase of the cell cycle. In the absence of those deacetylases (in cells that are Δhst3 Δhst4) there is hyper acetylation of H3K56 throughout the entire cell cycle and on both new and parental histones, conferring genomic instability that leads to phenotypes of defective growth and thermosensitivity.

Here we report that cells experiencing hyper-acetylation of H3K56 are dependent on the protein kinase Dun1 for survival, in a manner that is independent of Dun1's known role in upregulation of dNTP levels in the cell. The synthetic lethality of Δdun1 Δhst3 Δhst4 mutants is suppressed by mutations in the alternative clamp loader Ctf18, but not by mutations in the other alternative clamp loaders, Elg1 and Rad24.

We characterized this new regulatory pathway and found that it includes additional proteins involved in the S-phase checkpoint, such as the Mrc1 checkpoint adapter, as well as downstream targets of Dun1, such as Npl3. Like Ctf18, the E3 ubiquitin ligase Rtt101 is detrimental in Δhst3 Δhst4 cells and is under the regulation of the Mrc1-Dun1-Npl3 pathway. Neither Rtt101 nor Ctf18 interfere with the acetylation of H3K56, and act downstream of it. Importantly, Ctf18's detrimental effect in cells with hyper-acetylated histones is abolished by mutations that prevent its interaction with DNA Pol ε. Together, our findings argue that in the presence of hyper acetylated H3K56, Dun1, Mrc1 and Npl3 play a critical role in maintaining viability against harmful effects caused by the activity of Ctf18 and Rtt101.

53 High efficient homology-independent chromosome integration of foreign DNA fragment in Yarrowia lipolytica

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As an important oleaginous and industrial microorganism, Yarrowia lipolytica has been used to produce biofuels and other value-added compounds in the past few years. Although several genetic engineering tools have been developed in Y. lipolytica, the genome integration of DNA is still inefficient. In addition, it is also laborious and time-consuming to construct the libraries for gene expression and biochemical pathway optimization. In this study, we demonstrated that multiple and large DNA fragments can be randomly integrated into the chromosome with high efficiency through a homology-independent manner. The homology-independent integration can generate chromosome location variation and gene copy number difference, which will result in the expression variations of integrated genes or pathways. Based on these phenomena, we can create the gene expression libraries easily through one-step integration. further investigation revealed the high frequency and high efficiency of such integration. Our work provided a potential strategy for efficient DNA integration and library construction in Y. lipolytica.

54 Natural Variation and Heterogeneity in the Saccharomyces cerevisiae Lag Phase.

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In nature, microbes continuously encounter changes in nutrients and need to adapt to these changes in order to survive. During the necessary physiological reprogramming, cells can enter a so-called lag phase, during which cell growth can be completely arrested. Intuitively, you would expect that cells try to adapt as quickly as possible to a nutritional change so they can resume growth and compete with other organisms. However, lag phase length varies significantly between different Saccharomyces cerevisiae strains upon a shift from glucose to a secondary carbon source. In addition, some cells within an isogenic population take much longer to adapt than others. Which genetic factors cause this natural variation and heterogeneity in lag phase duration? And how does this affect the cells' physiology, growth and fitness in different environments?

Previously, we found that inducing carbon source-specific genes such as transporters and hydrolases, though necessary, is not the rate-limiting process determining lag phase duration upon a carbon source shift. Rather, re-routing the carbon flux from fermentation to respiration determines how fast cells adapt to a nutritional change [1,2]. Using bulk segregant QTL (Quantitative Trait Loci) analysis, we now identified a so-far uncharacterized gene that explains why some yeast strains can make a homogeneous, fast transition to a new carbon source, whereas others show highly heterogeneous long lag phases. In line with our previous results, this gene seems to be a regulator of respiration. Characterizing the precise molecular role of this gene will expand our knowledge on the mechanism behind the lag phase.

[1] Cerulus B, Jariani A, Perez-Samper G, et al (2018) Transition between fermentation and respiration determines history-dependent behavior in fluctuating carbon sources. Elife. doi: 10.7554/eLife.39234

[2] Perez-Samper G, Cerulus B, Jariani A, et al (2018) The Crabtree Effect Shapes the Saccharomyces cerevisiae Lag Phase during the Switch between Different Carbon Sources. MBio. doi: 10.1128/mbio.01331-18

55 Evolutionary Trajectories Reflect the Modularity in Genetic Networks.

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Genetic networks typically consist of modules with genes that are involved in related cellular processes. Intuitively, one would expect that this modular structure also affects how networks evolve over time. For example, strains lacking genes from the same module presumably would acquire mutations in similar pathways. Previous studies have indeed shown that the function of a gene is an important determinant during evolution but have only provided limited evidence for convergent evolution at the level of network modules. Here, we evolve Saccharomyces cerevisiae strains with deletions in specific modules of the genetic network underlying resistance to oxidative stress, a trait of both medical and industrial importance, to examine the effect of network modularity on evolution. We first determined the fitness of all ~4,800 strains in the yeast deletion collection under oxidative stress to identify the key genes involved in resistance to oxidative stress. Next, we constructed knock-out mutants of 50 key genes in different modules and evolved those sensitive mutants for 150 generations under oxidative stress conditions (paraquat). We then geno- and phenotyped the evolved strains to identify all compensatory mutations. We show that the outcome of evolution does not only depend on the original genotype, but that strains with deletions in different modules follow distinct mutational trajectories in a highly repeatable fashion. By identifying how defects in one module can be overcome by changes in another network module, these results not only tell us more about how different genotypes evolve, but also reveal the underlying structure of a complex trait.

56 Single Molecule Tracking Reveals Fast Interdependent Cycling of Transcription Factor Ace1p and Chromatin Remodeler RSC at CUP1 Promoter in Yeast.

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NCI/NIH

We investigate the molecular links between the dynamic binding of Transcription Factors (TF) and chromatin remodeling and transcription. For many dynamically bound TFs increase in their residence time at the specific sites improves the transcriptional output of the promoter. RSC chromatin remodeler affects the transcription of the yeast metallothionein-encoding CUP1. Using Single Molecule Tracking (SMT), we show that the chromatin remodeler RSC decreases the residence time of the TF, and speeds up the search process of the TF Ace1p for its Response Elements (RE) at the CUP1 promoter. Quantification by smFISH CUP1 mRNA data using a gene bursting model indicates that RSC regulates transcription bursts of CUP1 by modulating TF occupancy. Reduction in search time leads to more frequent bursts. Reduction in residence time reduces burst amplitude. SMT demonstrates that RSC binds to activated promoters transiently. Therefore, transient binding of Ace1p and rapid bursts of transcription at CUP1 may be dependent on short repetitive cycles of nucleosome mobilization. Based on smFISH modeling, this type of regulation reduces the transcriptional noise and ensures a homogeneous response of the cell population to heavy metal stress.

57 Anoxia-Induced Mitophagy in Kluyveromyces marxianus

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Mitochondrion is an organelle essential for oxidative ATP synthesis and its morphology changes dynamically responding to environmental conditions in yeasts. One of the mechanisms for mitochondria behavior is mitophagy, by which damaged and extra mitochondria are incorporated into vacuoles and degraded in autophagy-dependent manner. In Saccharomyces cerevisiae, prolonged incubation in a culture with a non-fermentable carbon source or shift to nitrogen starvation condition from the same type of culture induces mitophagy. In this study we show that mitophagy is induced by anoxia in the yeast Kluyveromyces marxianus..

K. marxianus strain expressing mitochondria-targeted GFP (mtGFP) was cultured in YPD medium in two different conditions. In atmospheric oxygen, mtGFP showed general dotted and tubular forms. Under anaerobic condition, however, GFP fluorescence was observed as a single large circular form. This localization change was not observed in S. cerevisiae. Visualization of vacuoles using FM4-64 in K. marxianus revealed that the large circular localization was vacuole. Vacuole is the organelle for degradation during autophagy. To reveal whether the localization change was caused by the autophagic process, mtGFP was expressed and observed in knockout mutants. Disruption of KmATG8 or KmATG32 prevented vacuolar localization of mtGFP under anaerobic condition. ATG8 is essential for all types of autophagy and ATG32 is specifically required for mitophagy. By electron microscopy, mitochondria-like membrane structures were observed in the vacuoles of the cells grown under anaerobic condition. Quantitative analysis was conducted using mitochondria-targeted Pho8, which is activated only when localized in the vacuoles. As a result, Pho8 activity was increased by anoxia treatment but not by nitrogen starvation which induces non-specific autophagy. From these results, we concluded that anoxia induced mitophagy in K. marxianus.

We also analyzed the effect of inhibitors against oxidative phosphorylation. Antimycin A and ferric cyanide, which inhibit electron transport, did not affect mtGFP localization under aerobic and anaerobic conditions. In contrast, CCCP, which depolarizes mitochondria membrane potential, inhibited mitophagy under anaerobic condition but not induce mitophagy under aerobic condition, suggesting that mitochondrial membrane potential is involved in anoxia-induced mitophagy in K. marxianus.

58 Study of Functional Modules Stability - Transcriptomic Analysis of Saccharomyces cerevisiae Genomes During the Laboratory Evolution.

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'Functional modules' are a group of genes or their products participating in the same biological process, which are linked by at least one genetic or physical interaction. An important property of a module is that its members have more relations among themselves than with members of other modules, which is reflected in the network topology.

Previous studies suggest that the loss of function of one of the genes from given module often leads to the inactivation of another gene from that module, which compensates the previous loss. Presumably, it is because deletion of a given gene causes module damage—so that the entire mechanism is malfunctioning and therefore it is preferable to remove other modular genes.

In our research we took a closer look at the dependencies between genes of functional modules related to NUP133 and COG7. Both of the genes have orthologues in other organisms and exhibit numerous genetic interactions. Nup133 is nuclear pore complex protein whereas Cog7 is a part of vesicular transport module.

The purpose of our research was to determine whether a lack of NUP133 or COG7, respectively, causes compensatory inactivation of other genes belonging to the tested modules. For that, we conducted experimental evolution of S. cerevisiae mutant strains in long-term cultures in chemostat. After c.a 200 generations evolved populations (and wild-type strain as a control) were subjected to genome sequencing for identification of changes resulting from each primal mutation. Our results indicate the emergence of a relatively small number of non-synonymous mutations in the whole yeast genome and probably only few compensatory mutations.

In both cases mutations in evolved populations in majority localized in probable promoter regions which indicate that the evolution of gene expression may play an important role in compensatory changes in cellular machinery after gene inactivation. So the compensatory effects could be exerted not only by affecting protein properties but also by affecting genes at the transcriptional level. To verify whether mutations in 5' upstream regions substantially influenced transcription, we conducted microarray analysis of the evolved yeast populations. Initial results indicate that 88 (in nup133Δ) and 21 genes (in cog7Δ) showed significant expression changes in comparison to evolved wild-type population.

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59 A Universal Tool for Identification of Superior Cell-factory Hosts in Genetically Diverse Yeast Species

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Yeasts have been widely used for construction of novel cell factories that can convert renewable biomass into different types of molecules such as biofuels, bulk, and fine chemicals. To this day, a vast majority of proof-of-concept studies are based on relatively small number of yeast species, most often limited to a few laboratory isolates of well-characterized Saccharomyces cerevisiae. Yet, only a very small fraction of those have been developed to reach commercially feasible product yields due to high costs associated with iterative strain optimization cycles. One-way to overcome this problem could be identification of expression host readily equipped with a most optimal genetic landscape for production of a given molecule. In fact, an accumulating number of studies indicate that production yields can vary significantly within strains of the same yeast species. The latter leads to the fact that there is unexploited potential in the biodiversity of available yeast for construction of superior cell-factories.

Here we present a novel tool, DIVERSIFY, that facilitates quick and easy screening for production capabilities of any compound of interest in evolutionary distant yeast species. We have developed the DIVERSIFY strain collection harboring a multi-functional gene-targeting cassette integrated into their genomes. The cassette contains standard gene-targeting sequences interspaced with I-SceI restriction site, dominant marker and chromogenic marker that enables easy screening for correct recombination. In addition, DNA double strand brake can be induced by the I-SceI meganuclease to promote gene targeting. In this study we demonstrate that DIVERSIFY platform allows quick and efficient marker-free gene transfer into broad range of different yeast species.

60 Hexokinase and Glucokinases Are Essential for Fitness and Virulence in the Pathogenic Yeast Candida albicans

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The pathogenic yeast Candida albicans is both a powerful commensal and a pathogen of humans that can infect wide range of organs and body sites. Metabolic flexibility promotes infection and commensal colonization by this opportunistic pathogen. Yeast cell survival depends upon assimilation of fermentable and non-fermentable locally available carbon sources. Physiologically relevant sugars like glucose and fructose are present at low levels in host niches. However, because glucose is the preferred substrate for energy and biosynthesis of structural components, its efficient detection and metabolism are fundamental for the metabolic adaptation of the pathogen. We explored and characterized the C. albicans hexose kinase system composed of one hexokinase (CaHxk2) and two glucokinases (CaGlk1 and CaGlk4). Using a set of mutant strains, we found that hexose phosphorylation is mostly performed by CaHxk2, which sustains growth on hexoses. Our data on hexokinase and glucokinase expression point out an absence of cross regulation mechanisms at the transcription level and different regulatory pathways. In the presence of glucose, CaHxk2 migrates in the nucleus and contributes to the glucose repression signaling pathway. In addition, CaHxk2 participates in oxidative, osmotic and cell wall stress responses, while glucokinases are overexpressed under hypoxia. Hexose phosphorylation is a key step necessary for filamentation that is affected in the hexokinase mutant. Virulence of this mutant is clearly impacted in the Galleria mellonella and macrophage models. Filamentation, glucose phosphorylation and stress response defects of the hexokinase mutant prevent host killing by C. albicans. By contributing to metabolic flexibility, stress response and morphogenesis, hexose kinase enzymes play an essential role in the virulence of C. albicans.

Laurian et al., 2019. Frontiers in Microbiology, volume 10, article 327.

61 Using Metagenomic Genes to Engineer Saccharomyces cerevisiae Strains for Improved Tolerance to Acid Conditions.

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Biomass conversion to biofuels and added-value chemicals has been largely encouraged in order to move to a more sustainable economy. Recent innovations in obtaining products from plant biomass degradation include the improvement of microorganisms and enzymes capable of degrading such materials. The idea consists in obtaining organisms capable to breakdown cellulose and ferment the resulting sugars while tolerating high concentrations of product, osmotic pressure and ion toxicity, temperature, toxic compounds from lignocellulose pretreatment and low pH, for instance. Engineering fermenting microorganisms to degrade lignocellulosic biomass and survive in harsh conditions during a consolidated-bioprocess can be crucial for reaching economically viable processes. In this sense, metagenomic findings appears as a relevant toolbox for yeast engineering by providing novel enzymes, promoters and genes that confer resistance to extreme conditions with potential use for industrial applications. In this work, we have used metagenomic genes (recovered from functional metagenomic screenings) that conveyed acid tolerance in E. coli and other gram-positive and gram-negative bacteria to engineer laboratorial and industrial Saccharomyces cerevisiae strains. By using integrative vectors, we inserted metagenomic genes that display putative functions of a protease and of a histone-like protein in two S. cerevisiae strains: a haploid laboratory strain and a diploid industrial bioethanol producer strain, generating four engineered strains. All the metagenomic genes were inserted in chromosomal sites that provide high protein expression levels, as well as all the genes are under control of a constitutive promoter (native of S. cerevisiae). Engineered strains will be submitted to acid-shock experiments and the capability of the genes to confer acid resistance to S. cerevisiae will be verified by comparing mutant and control surviving rates. Our ultimate goal is to apply the engineered yeast strains on small-scale fermentation processes. The results should provide new directions for use of genes obtained from metagenomics approaches to expand the toolbox for engineer microorganisms acting in consolidated-bioprocesses.

62 Measuring and Modelling Yeast Colony Growth.

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Colonies built by natural isolate strains of the budding yeast, Saccharomyces cerevisiae show various morphologies. To understand the differences in the growth behaviour of these strains we study the quantitative features of yeast colonies. Our major focus is on the laboratory strain BY4743 and the vineyard isolate M281-B. The later one forms filigreed / fluffy colonies. We quantify the number of the cells, the density, the size and the shape of the colonies initiated from various inoculation scenarios to reveal how the density of the colony affects the proliferative capacity of the cells, both in the yeast and the filamentous growth modes. These quantitative data is used to train our agent-based mathematical model that is capable of capturing growth differences of various yeast strains. The properly parameterised model is helping us to design synthetic yeast co-colonies with desired layouts.

63 Cellular Interactions in Yeast Colonies

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After the more thorough understanding of the intracellular processes, nowadays more and more researchers are interested in understanding the interactions occuring between cells. During our experiments, we focus on examining the interactions that arise, in our case, between yeast cells. This is important because in their natural environment yeast strains grow in mixed colonies, while in most laboratory experiments of microbial colony growth researchers are focusing on single strains. The most common interaction that occurs in every microbial colony is the competiton for nutrients, but besides this microbes can develop many strategies to survive, like cooperating with each other or producing toxins against each other. In order to get a bigger picture about these interactions and their molecular background, we examine these interactions between different natural strains of the budding yeast Saccharomyces cerevisiae. In order to differentiate the strains in a mixed colony we use two fluorescent proteins EGFP and mCherry to label our strains. By measuring the growth of our candidate strains in mixed colonies, our preliminary results show that some of the investigated strains show so far uncharacterised interactions. Our main goal is to determine how such interactions between different strains influence the future of mixed colonies. Along with the laboratory experiments an agent-based model is being developed to simulate the growth of single and mixed colonies. Because of this, our laboratory experiments are also used to provide better parameters for the model.

64 Degradation of Integral Membrane Proteins Modified with the Ubiquitin-independent Photo-sensitive Degron Module Require Ubiquitylation and the Cytosolic Endoplasmatic-Reticulum Associated Degradation Pathway.

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Protein quality mechanisms are fundamental for proteostasis of eukaryotic cells. The endoplasmatic reticulum associated degradation (ERAD) is a well-studied pathway that scans secretory and endoplasmatic reticulum (ER)-resident proteins for malfolded specimen. Different branches of ERAD are involved in degradation of malfolded secretory proteins, depending on the localization of the misfolded part, the ER lumen (ERAD-L), the ER membrane (ERAD-M), and the cytosol (ERAD-C). Here we report that modification of several ER transmembrane proteins with the photo-sensitive degron (psd) module resulted in light-dependent degradation of the membrane proteins via the ERAD-C pathway. Surprisingly, the ubiquitin-independent photosensitive degron that is usually directly recognized by the proteasome , is now requiring ubiquitylation for degradation and is not recognized solely by proteasomes when fused to an ER membrane protein. This change in the degradation mechanism for the psd module while attached to a membrane protein might indicate a similarity between substrates directly recognized by the proteasome and the protein quality control systems. Thus, our work shows that ERAD-C substrates can be systematically generated via synthethic degron constructs, which facilitates future investigations of the ERAD-C pathway.

65 Haploinsufficiency Mechanisms and Evolution.

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Genomic structural variations (e.g. deletions, duplications) are common in the human genome. Each individual carries at least 200 genetic variants predicted to severely disrupt protein-coding genes, collectively known as loss of function variants. These deletions cause diseased phenotype in some, but not all individuals. Why? One possible explanation can be that healthy individuals have accumulated modifier mutations elsewhere in the genome that can mask the aberrant phenotype of the affected gene. Recent theoretical studies have indicated that modifiers of dominance can be favored by selection in certain circumstances, but the nature of such modifiers has remained largely elusive. To investigate this issue systematically, we performed laboratory evolution with 184 diploid heterozygotes knock-out baker's yeast strains all of which displayed reduced fitness in the laboratory (i.e. haploinsufficient). 44,4% of the parallel evolving populations displayed increased fitness in stress-free conditions, suggesting that the evolution of dominance modifiers is pervasive. Genomic analysis revealed that chromosomal duplications and point mutations elsewhere in the genome are responsible for the elevated fitness of haploinsufficient strains. However, these genetic changes have associated fitness costs in specific environmental settings. In sum, dominance modifiers are ubiquitous and have a wide-range of pleiotropic side effects.

66 Distinct patterns of cell death and division arrest at early replicative ages revealed by genome-wide screening

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Cell death and its deregulation are central features of aging, cancer and various degenerative diseases in humans. Understanding cell death in microorganisms is also highly relevant for the development of drugs as well as for a basic understanding of how living systems function and break down. In this work, we have used Saccharomyces cerevisiae to screen for genetic perturbations that increase the death rate of yeast cells on rich medium with glucose as a carbon source. Using phloxine, a non-toxic die that does not penetrate cells with intact membranes, but effectively stains dead cells, we assayed the full yeast knockout collection as well as a collection of strains with downregulated essential genes. This screen identified 127 genes, about 2/3 of them being essential. We confirmed these results by flow cytometry of propidium iodide-stained cells. Interestingly, phloxine staining of colonies revealed different patterns of cell death – some mutants were more prone to die at the edge of the colony, others were stained uniformly, and others preferentially died at the colony center. This suggests different sensitivity of the mutants to their environment, such as contact with the medium, starvation, and high density growth. To determine the replicative age at which the cells were most likely to die, we developed the DIVision Arrest assay (DIVA), which involves staining of cell walls with a fluorescent agent, doubling of the culture ~10 times in order to age the stained cells, and quantification of budscars of the stained cells. DIVA allows detection of cells that stopped dividing at early replicative ages, as compared to the majority of stained cells. This assay, together with several others, showed that a considerable number of phloxine-positive mutants exhibited rapid cell permeabilization at early replicative age. Interestingly, we also observed an early-age division arrest in some phloxine-negative strains which were randomly selected from strains with downregulated essential genes. Our data represent the first systematic study of genes whose deficiency increased the chance of early cell death. It also identifies distinct death patterns in yeast mutants, suggesting different mechanisms of cell death, and demonstrates division arrest at early ages in a large number of strains.

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67 Compensatory Mutations Drive Morphological Evolution

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Microbes display great diversity in cellular morphology. Traditional explanations of this variation are adaptation to changing environments and neutral evolution. Here we propose that strongly deleterious mutations can also contribute to morphological diversity by generating selection pressure for compensatory evolution which may lead to alternative morphological states. We tested this scenario with more than 100 haploid budding yeast knockout strains - representing diverse disrupted gene functions - which underwent compensatory evolution in the laboratory. We carried out high-throughput automated microscopy and image analysis on the ancestor and evolved lines of knock-out strains, allowing us to investigate hundreds of single-cell attributes. We found that the restoration of wild-type cellular morphology was not prevalent. Instead, numerous lines diverged both from the wild-type and their corresponding ancestor, and thereby evolved novel cellular morphology. Strikingly, not only cellular morphology, but also other phenotypes changed during compensatory evolution. Specifically, some of the strains gained the ability to invade solid surfaces, which is an important virulence factor of pathogenic fungi. Taken together, deleterious mutations can promote the evolution of cellular morphology and may contribute to the emergence of fungal pathogenicity.

68 Genome Stability, Karyotype Plasticity, DNA Damage Response, and Morphogenetic Switching in the Fungal Pathogen Candida auris

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Candida auris has been known to science only for a decade, when it was isolated from the ear canal of a patient in Japan. Since then it has developed into a major health concern all over the world. It is one of the most drug-resistant yeasts and its emergence and population structure are unusual in many respects. Due to its recent emergence, the general biology and life cycle of this fungus is largely unexplored. Because of the evolutionary distance to the best-studied Candida species C. albicans, inferences from research on C. albicans are not transferable to C. auris. Indeed, research by us and others indicate that these two fungi behave very differently regarding their cell biology.

The ability to undergo morphogenetic switches between different growth forms (yeast and hyphae) is a key virulence factor in C. albicans. However, most of the cues causing filamentation in C. albicans do not have this effect in C. auris. Intriguingly, our results show that most, but not all, of the 22 clinical isolates of C. auris tested produce pseudohyphal filaments under genotoxic stress or in the absence of functional Homologous Recombination (HR). C. auris is able to produce filaments when challenged with genotoxic drugs, or when RAD51 or RAD57 are deleted (VPCI479/P/13 strain background). Triggering the S-phase checkpoint will be discussed as a cause for these observations. Salt-stress and passage through a mammalian host also prime this yeast for pseudohypha formation (Wang et al. 2018, Emerg Microbes Infect 7:93; Yue et al. 2018, Emerg Microbes Infect 7:188) indicating that morphogenetic switching in C. auris is complex, and will likely be caused by multiple cues.

Our studies also show that clinical C. auris isolates differed considerably in chromosome numbers and sizes, both within and between geographical clades, which was unexpected considering the genetic uniformity of C. auris on a DNA sequence level within geographical clades. When this yeast is put under stress, it can undergo marked alterations within a short time-frame. Intriguingly, adaptation to osmotic stress can induce resistance to the clinically-used antifungal drug Caspofungin. This indicates that gross chromosome rearrangements might be a mechanism C. auris employs to generate genetic diversity during adaptation to environmental challenges.

69 Mechanisms of antimony genotoxicity in budding yeast.

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Antimony is a toxic metalloid present in the environment from both natural and anthropogenic sources. In humans, chronic exposure to antimony causes numerous diseases, including cancer. On the other hand, because of its toxic properties antimony is used in the treatment of tropical diseases caused by the protozoan parasites and antimony-based compounds show promising anticancer properties in pre-clinical trials. Antimony is classified as a possibly carcinogenic element. However, little is known about mechanisms of its cytotoxicity or genotoxic potential. Here, we aimed to elucidate how and what type of DNA damage is induced by antimony using budding yeast as a model organism. We found that antimony activates DNA damage checkpoint as shown by Mec1 and Tel1-dependent phosphorylation of histone H2Aand Rad53 leading to cell cycle delays in S and G2/M phases. We also observed induction of DNA damage checkpoint in G1-synchronized cells devoid of Yku70 suggesting that antimony is capable of inducing double-stranded DNA breaks similar to related metalloid arsenic. Moreover, elevated levels of Rfa1 and Rad52 foci were formed in the presence of antimony and both checkpoint and homologous recombination mutants showed increased sensitivity to this metalloid. However, in contrast arsenic, we were not able to detect formation of DSBs by PFGE during exposure to antimony. We did observe low-levels of antimony-induced oxidative DNA damage as well as accumulation of single-stranded DNA gaps suggesting damage-related replication perturbations. Interestingly, we noticed that mutations in genes involved in the telomere maintenance are particularly sensitive to antimony leading to a hypothesis that antimony causes alterations in telomere regions and/or interferes with the telomere-associated proteins leading to DNA damage checkpoint activation and genomic instability. Indeed, we found that antimony affects levels of Cdc13, Rap1 and Ku70 at telomeres and increases telomere fusions as well as chromosome loss but does not affect length of telomeres. In sum, we conclude that antimony exhibits clear genotoxic properties in the yeast model and its carcinogenic potential should be re-evaluated.

70 Regulation of Glucose Metabolism Through Hog1 in Response to Osmostress.

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Stress Activated Protein Kinases (SAPKs) are key players regulating the cellular response upon environmental changes. The exposure to environmental changes triggers the activation of a high conserved SAPKs family, including Hog1 in yeast and p38 in mammals. The HOG (High Osmolarity Glycerol response) pathway in yeast has been investigated in depth and the high functional conservation among species has allowed a better understanding of the p38 pathway in mammals. It is known that Hog1 regulates cell cycle, gene expression and the intracellular glycerol levels during osmostress. To unravel novel functions of Hog1, we did a systematic genetic and chemical approach and identified new proteins that can be potentially targeted by Hog1. One of this proteins is Phosphofructokinase 27 (Pfk27) that, together with Pfk26, generates Frustose-2,6-biphosphate from Fructose-6-phosphate. The product of this reaction acts as an allosteric activator of Pfk1, a key enzyme of glycolysis. We are characterizing the molecular mechanism underlying the regulation of Pfk27 and its role in glucose metabolism during osmostress.

71 Unusual DNA Binding Preferences of the Yeast AP-1-like Transcription Factor Yap8 Are Dictated by the N-terminal Ancillary Region Adjacent to the Basic Region.

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Activator protein 1 (AP-1) is one of the largest families of basic leucine zipper (bZIP) transcription factors in eukaryotic cells. How AP-1 proteins achieve target DNA binding specificity remains elusive. In Saccharomyces cerevisiae, the AP-1-like protein (Yap) family comprises eight members (Yap1 to Yap8) thatdisplay distinct genomic target sites despite high sequence homology of their DNA binding bZIP domains. In contrast to the other members of the Yap family, which preferentially bind to short (7-8 bp) DNA motifs, Yap8 binds to an unusually long DNA motif (13 bp). It has been unclear what determines this unique specificity of Yap8. In this work, we use molecular and biochemical analysis combined with computer-based structural design and molecular dynamics simulations of Yap8-DNA interactions to better understand the structural basis of DNA binding specificity determinants. We identify specific residues in the N-terminal tail preceding the basic region, which define stable association of Yap8 with the ACR3 promoter. We propose that the N-terminal tail directly interacts with DNA and stabilizes Yap8 binding to the 13 bp motif. Thus, beside the core basic region, the adjacent N-terminal region contributes to alternative DNA binding selectivity within the AP-1 family.

72 Impact of Mitochondrial Proteome on the Evolution of Yeast Hybrids.

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Interspecific hybrids of the Saccharomyces sensu stricto species inherit a bi-parental genome but retain the mitochondrial DNA of only one parent. In this environment, where the majority of the mitochondrial-related proteins are encoded by the nucleus, the potential to form chimeric protein complexes may confer improved properties to the hybrid strains. However, cases of hybrid defects and lethality have been previously attributed to cytonuclear incompatibilities, a phenomenon also associated with severe cellular dysfunctions and degenerative diseases in humans. In this study, we aim to investigate the role of mitochondria in the evolution of hybrid genomes by exploring how the different abundance of mitochondrial-associated proteins may influence the dynamics of the mitochondrial proteome in hybrids of S. cerevisiae with S. uvarum. To address this, we used the Fluorescence Correlation Spectroscopy (FCS) microscopy approach to first generate a reference set of absolute quantitative data for the low-abundant fission protein, Fis1p, in intraspecific S. cerevisiae and S. uvarum hybrid strains growing under respiratory conditions. Preliminary FCS data in the S. cerevisiae parental strains growing in respiratory conditions and at 30oC reveal the absolute concentrations of GFP-labelled Fis1p to be of ~88-110 molecules per cell. Moreover, FCS measurements on the Fis1p mobility provide evidence that the protein is mainly found free in the cytoplasm of hybrid cells growing in respiratory conditions. Further comparison of the FCS-based molecule concentrations to the data generated in the hybrid environment with either S. cerevisiae or S. uvarum mitotype will provide us an absolute quantification of single-cell protein-protein interactions. This will help us better understand how protein dynamics change throughout cellular functions and further enhance ongoing attempts to model protein pathways and define global protein abundance in yeast.

73 Snf2-like Protein Irc5 Facilitates Error-free DNA Damage Tolerance Pathway Through Cohesin.

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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 have several other functions including DNA repair. Here we report that Irc5, a member of the Swi2/Snf2 ATPase family, is a novel interactor of cohesin that enables cohesin binding to chromosomes by promoting efficient cohesin loading. We also show that during replication stress Irc5 promotes cohesin accumulation at stalled replication forks facilitating efficient formation of sister chromatid junctions that are crucial for error-free DNA lesion bypass. This prevents accumulation of ssDNA gaps and allows replication completion. Our results support the notion of a key role of cohesin in the completion of DNA synthesis under replication stress and reveal that the Rad18/Rad5-mediated DDT pathway is linked to cohesin enrichment at sites of perturbed replication via the Snf2 family translocase Irc5.

74 Turnover and Substrate Specificity of the Arsenite Transporter Acr3 from Budding Yeast.

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Uniwersytet Wroclawski

The yeast plasma membrane transporter Acr3 mediates efflux of toxic arsenite and antimonite. We investigated the mechanisms of Acr3 turnover. We found that after arrival and residence at the plasma membrane, Acr3 is subjected to Rsp5-dependent ubiquitination and internalization followed by proteolysis in the vacuole. We found that a short acidic patch located in the N-terminal tail of Acr3 is needed for its ubiquitination and internalization. We propose that this motif serves as an endocytic signal that facilitates binding of the arrestin-Rsp5 complexes to the Acr3 cargo. Members of the Acr3 family display different substrate specificities; for example Acr3 proteins from Corynebacterium glutamicum or Alkaliphilus metalliredigens confer only arsenite resistance and are not able to transport antimonite. Using random and site-directed mutagenesis approaches, we identified two conserved hydrophobic residues located in transmembrane regions of Acr3 that contribute to substrate specificity. Mutation of Val155 residue resulted in a loss of arsenite transport and sensitivity to arsenic, but not to antimonite. In contrast, mutation of Val173 residue strongly impaired antimonite transport activity of Acr3. We propose that both residues may influence the overall structure of Acr3 within the plasma membrane as well as shape the substrate-binding site.

75 Towards Establishing a Method to Measure Translation in Pichia pastoris.

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Protein synthesis is regulated at several cellular levels, starting with transcription of the gene of interest and subsequent translation of the mRNA. To better understand cellular processes of protein production, rate measurements at limiting steps are necessary. While abundant methods are available to measure transcription levels, measuring translation is still a challenge. In recent years, the antibiotic puromycin has been used as a possible method. Puromycin incorporates itself into nascent polypeptide chains, making measurement of translation rates possible. So far, yeasts, specifically Saccharomyces cerevisiae, have proven to be problematic as they are resistant to puromycin.

Hitherto Pichia pastoris (syn. Komagataella spp), a yeast commonly used for recombinant protein production, was not tested for susceptibility to this antibiotic. Interestingly, in the P. pastoris genome we did not find two out of three gene homologs responsible for conferring puromycin resistance in S. cerevisiae. We tested two strains of P. pastoris with and without two single gene knockouts to ascertain said susceptibility to the antibiotic. For this we performed experiments to determine the minimum inhibitory and fungicidal concentrations. Additionally we identified the incubation time needed to inhibit cell growth, equalling the incorporation time.

We found puromycin inhibits growth of P. pastoris if strains with a single gene knockout are used. Therefore we conclude that measurement of overall translation rates should be possible. This work opens the way towards establishing a valuable tool for observation and manipulation of yeast cells during protein production.

76 Two Different Strategies to Engineer Yeast for Production of Aromatic Compounds

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We will present two alternative strategies for the synthesis of valuable aromatic compounds with the yeast Saccharomyces cerevisiae. For this, we either used the aromatic amino acid pathway to produce mandelic or 4-hydroxymandelic acid (1,2), and we used polyketide synthases to produce m-cresol (3). Mandelic and 4-hydroxymandelic acid are valuable specialty chemicals used as precursors for flavors as well as for cosmetic and pharmaceutical purposes. m-cresol (3-methylphenol) is utilized as disinfectant, act as an antioxidant scavenging reactive oxygen species, and is an important platform compound for synthesis of several chemicals with high market value like menthol.

For the production of mandelic or 4-hydroxymandelic acid (more than 1 g/L) we expressed and engineered heterologous hydroxymandelate synthases and optimized the flux into and through the phenylalanine or tyrosine branches, respectively, of the aromatic amino acid pathway. For the production of m-cresol (more than 0.5 g/L) we expressed the polyketide synthase methylsalicylic acid synthase together with a methylsalicyl acid decarboxylase. Limiting factors for the production of the different compounds were determined to be either the competition between mandelic and 4-hydroxymandelic acid production, or the toxicity of m-cresol.

(1) Metab Eng. 2018;45:246-254. doi: 10.1016/j.ymben.2018.01.001.

(2) Metab Eng Commun. 2018;7:e00079. doi: 10.1016/j.mec.2018.e00079.

(3) Hitschler J and Boles E, 2019. De novo production of aromatic m-cresol in Saccharomyces cerevisiae mediated by heterologous polyketide synthases combined with a 6-methylsalicylic acid decarboxylase. Metab Eng Commun. (accepted, in press).

77 Uncovering Targeting Priority to Peroxisomes using a new Targeting Competition Assay.

Mira Rosenthal, Eyal Metzl-Raz, Naama Barkai, Maya Schuldiner and Einat Zalckvar

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Organelles create biochemically distinct compartments enabling diversification of the cellular landscape. Each organelle harbors tens if not hundreds of proteins and subsets of these are more central to organelle function while others may be less so. Changes in environment and the needs of the cell dynamically determine which proteins will be targeted at which abundance to which location. To date, there is much known about the basal machinery enabling protein targeting to organelles, while still little is known about protein targeting priority - whether a mechanism exists that allows organelles to discriminate between different proteins to ensure that certain subsets of them are assured entry under specific conditions.

In this work, we used the yeast S. cerevisiae as a model to study if and how protein targeting is prioritized. The organelle of choice was the peroxisome, which plays a central role in cellular metabolism by breaking down fatty acids and detoxifying reactive oxygen species. Since peroxisomes are small organelles with a defined protein content they serve as a robust model organelle for such questions. To study protein targeting priority to peroxisomes we created a competition system that is based on expressing different levels of a mCherry protein with a strong Peroxisome Targeting Signal (PTS1) and systematically examining how it affects the targeting of all other known peroxisomal proteins, each tagged with GFP in its N terminus (N') thus leaving the C' PTS1 signals unperturbed.

We found that the mCherry-PTS1 competitor affected mostly the localization of peroxisomal proteins that are known to have a PTS1 signal and so use the same targeting pathway to be targeted to the peroxisomal matrix. Hence, membrane spanning proteins and other matrix proteins, who are targeted via a different targeting receptor, were not affected in localization. Interestingly, we found that within the PTS1 matrix proteins there was a subgroup of six proteins who did not show a change in localization when competing with the mCherry-PTS1 competitor. We suggest that that there is a targeting priority for these six proteins. We are now following up on the proteins with the highest priority to understand the factors in both cis and trans that endow them with this capacity.

Our unique system showcases how targeting priority can be systematically studied, deepening our understanding on protein targeting to peroxisomes as a model system to organelle protein targeting.

78 Rif2-mediated Regulation of MRX Activity at DNA Double-Strand Breaks

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DNA double-strand breaks (DSBs) are among the most cytotoxic DNA lesions, because failure to repair them can lead to genome instability. DSBs can be repaired by either nonhomologous end joining (NHEJ), which directly ligates the two broken DNA ends, or homologous recombination (HR), which uses an intact homologous DNA sequence as a template for repair. The key process in determining which pathway is used to repair DSBs is the initial processing of the DSB ends. While NHEJ requires little or no DNA end processing, HR is initiated by nucleolytic degradation of the 5' terminated strands at both DNA ends by a concerted action of nucleases in a process termed DNA end resection. The Mre11-Rad50-Xrs2/NBS1 complex (MRX in budding yeast, MRN in humans) has structural and enzymatic activities to initiate DSB resection and to maintain the DSB ends tethered to each other for their repair. Several studies have shown that ATP binding and hydrolysis activities of the Rad50 subunit are crucial to regulate DNA binding, tethering and nuclease functions of the MRX complex.

In budding yeast, MRX is known to interact with Rif2, which is recruited to telomeric DNA ends and negatively regulates telomerase-mediated telomere elongation. We have previously shown that Rif2, which is recruited to DSBs in a manner partially dependent on MRX, enhances the ATP hydrolysis activity of Rad50 and attenuates MRX function in end-tethering. This observation, together with the finding that the lack of Rif2 by itself increases both end-tethering and NHEJ, suggests that Rif2 can regulate MRX activity at DSBs by modulating ATP-dependent conformational changes of Rad50. To better understand the crosstalk between Rad50 and Rif2, we have searched for rad50 mutants that phenocopy RIF2 deletion and therefore that increase both NHEJ and end-tethering. We identified a mutation in Rad50 that is located on the surface of the protein, suggesting that it can affect a possible Rif2-Rad50 interaction. We will present data regarding the structural and functional characterization of this Rad50 mutant variant.

79 Non-Saccharomyces Wine Strains Face Problems during Biomass Propagation Due to Low Invertase and Stress Tolerance.

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In the winemaking industry, the use of active dry yeast (ADY) as inoculum is a well-established practice, due to its long-term stability. During industrial biomass propagation, yeast cells undergo a series of stresses, oxidative stress being the main one, which affect biomass yield and fermentative capacity of ADY. Recently, there has been an increasing interest in using non-Saccharomyces wine yeasts as inoculums in mixed fermentations, and there are already commercially available strains. Saccharomyces cerevisiae is well adapted to the biomass propagation process. However, some non-Saccharomyces species show defective growth in these conditions, mostly due to their lower tolerance to oxidative stress. Two non-Saccharomyces species Hanseniaspora vineae and Metschnikowia pulcherrima, which show distinct behaviour in ADY production tests, were tested. We observed differences in their oxidative stress defence mechanisms that correlate with their performance in ADY production conditions. We have detected that both species are incapable of fully depleting the sucrose present in molasses, linked to their low invertase activity. Acid hydrolysis with HCl allowed full hydrolyzation of sucrose permitted cells to consume all the available sugars. Our results validate that molasses hydrolyzation prior to growth in bench-top and bioreactor trials can have a beneficial effect on biomass yield and fermentative capacity of non-Saccharomyces ADY. In order to find new strains useful for their use as starters, yeasts isolated from traditional Ecuadorian chichas were tested for stress tolerance and growth on molasses. Three S. cerevisiae strains and one Torulaspora delbrueckii were selected for their good behavior, and with this strains, dehydration tolerance and invertase activity were analyzed. Regarding S. cerevisiae strains, significantly different growth in molasses was observed between strains while fermentative capacity was similar than controls; these strains also showed again a higher invertase activity compared to T. delbrueckii.

80 Direct From Nature – Novel Xylose Transporters And Molecular Mechanisms For Improved Sugar Uptake In Engineered Saccharomyces cerevisiae.

João Bueno, Guilherme Borelli, Juliana José, Thamy Corrêa, Gonçalo Pereira and Leandro Santos

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The need to restructure the world energy matrix based on fossil fuels stimulated the development of new technologies based on renewable energy. One promising and cleaner alternative towards energy restructuration is the second-generation (2G) fuels, produced from lignocellulosic biomass. A major challenge on 2G technology establishment is the inefficient assimilation of the five-carbon sugar xylose by engineered Saccharomyces cerevisiae strains, increasing the fermentation time. The xylose uptake by S. cerevisiae strains occurs by endogenous sugar transporters which have low affinity to xylose and strong glucose repression, impairing fermentation. By assessing microbiomes such as the digestive tract of plague insects or several biomasses, we isolated several yeast species capable of using xylose. Comparative fermentations select the yeast Candida sojae as a potential source of high-affinity transporters. Therefore, comparative genomic analysis elects four xylose transporters - Cs186, Cs2608, Cs3894, and Cs4130 - whose performance properties were evaluated in the transporter-free EBY.VW4000 strain carrying the xylose pathway integrated into the genome. While the traditional xylose transporter GXF1 is inhibited at concentrations above 10 g/L, the strains containing Cs3894 and Cs4130 show superior xylose uptake, not affected at concentrations up to 50 g/L. The modelled structure of Cs4130 shows the typical fold of membrane transporters belonging to the Major Facilitator Superfamily (MFS) with the extracellular, 12 transmembrane segments and intracellular domains. The superimposition with a notorious glucose/xylose transporter (XylE) structure harboring a D-xylose as ligand shows that the tyrosine 324 (Y324) residue in Cs4130 is dislocated when compared to GFX1, adopting an orientation that could act trapping the xylose molecule, preventing the scape to the extracellular side. Considering that xylose concentrations in 2G hydrolysates reaches high values, Cs4130 are a profitable candidate for xylose uptake due to natural loss of inhibition. Here we demonstrate a novel eukaryotic transporter protein that are not inhibited in high xylose concentrations which can be used as promising target towards efficient pentose utilization in engineered yeasts.

81 Flocculation of Saccharomyces cerevisiae is Dependent on Activation of Slt2 and Rlm1 Regulated by the Cell Wall Integrity Pathway.

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Flocculation is an essential characteristic of yeast cells required for survival under adverse conditions. The multicellular structure (flocs) of yeast provides a suitable microenvironment to enhance the chances of survival during stress conditions. Although the signaling events triggering flocculation have been studied earlier, molecular mechanism remained elusive. In the present study, we used flocculating sen1 mutants to identify the mechanism of flocculation. Based on the abnormal cell surface morphology and constitutive phosphorylation of Slt2 in flocculating Sen1 mutant cells, we assumed that flocculation is regulated by the Cell Wall Integrity (CWI) pathway. Up-regulation of FLO genes in wild type cells was observed upon activation of the CWI pathway either by chemical treatment or by deleting Slt2 phosphatase (Msg5). By using Slt2 mutants our studies reveal that the active state of Slt2 is indispensable for flocculation. The flocculation was reduced after deletion of SLT2 or RLM1. Further, we revealed overlapping binding sites for Rlm1 and Tup1 at the promoters of almost all the FLO genes. Finally, we show higher occupancy of Rlm1 and lower of Tup1 at the promoters of FLO1 and FLO5 in flocculating cells. Altogether we demonstrate that CWI MAPK (Slt2) pathway use a non-catalytic mechanism to activate the transcription of FLO genes.

82 Genetic Dissection of the Functional Relevance of Protein Phosphorylation

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Protein phosphorylation is a reversible signaling mechanism involved in all cellular processes. Phosphorylation has been shown to diverge rapidly during evolution suggesting that some phosphorylation sites may not be functional. Over 20,000 phosphorylation sites have been identified in S.cerevisiae, however, for the majority of them the function and the extent by which they contribute to fitness remains unknown. Here, we constructed a library of 500 phosphodeficient mutants across 130 genes covering many cellular processes. We then carried out a high throughput chemical genetic screen of our library, in conjunction with the yeast KO collection across 100 stress conditions. Our screen shows that 46% of the phosphodeficient mutants show at least one conditional growth phenotype. By generating a phenotypic fingerprint for each individual mutant we were able to compared each phosphodeficient mutant with each gene KO and assign novel functional associations.

83 Ixr1 is an intrinsically disordered protein with prion-like characteristics

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CICA-Universidade da Coruña

Ixr1 is a transcriptional regulatory factor of Saccharomyces cerevisiae previously identified as a player in the response to several stress conditions, such as oxidative stress or hypoxia, as well as in the resistance to cisplatin treatment (Vizoso-Vázquez et al., 2018). Our lab have previously demonstrated that the amino-terminal part of this protein is involved in the transcriptional activation (Barreiro-Alonso et al., 2018). Nevertheless, little is known about the structure and mechanism of action of this protein.

Here, we show that Ixr1 is an intrinsically disorder protein with high tendency to aggregate, displaying large disorganized regions flanking the HMG boxes which conform the DNA binding domains. Indeed, Ixr1 aggregation is highly ordered and the protein is able to form amyloids. Amyloid fibrils are one of the most frequent self-templating replicative states among the prions characterized until now. The relationship between Ixr1 function and its prion capabilities is also analyzed and discussed.

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- Vizoso-Vázquez Á, Lamas-Maceiras M, González-Siso MI, Cerdán ME. (2018) “Ixr1 Regulates Ribosomal Gene Transcription and Yeast Response to Cisplatin.” Scientific Reports 8(1):3090.

84 Understanding the Temporal Regulation of Metabolism in Yeast.

Helen C. Causton, John O'Neill, Nathaniel Hoyle, J. Brian Robertson, Rachel Edgar, Andrew Beale, Sew Y. Peak-Chew, Jason Day, Sofia da Costa and Christian Frezza

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Yeast respiratory oscillations (YROs), are metabolic rhythms that share diverse features with circadian rhythms, suggesting they either represent an ancient and conserved phenomenon that is likely to be driven by mechanisms common to all aerobic eukaryotes, or draw on general metabolic functions. The YRO temporally coordinates a wide range of cellular processes and is highly amenable to systems-wide analysis. We have generated a comprehensive description of metabolites, ions and proteins in yeast undergoing oscillations of different periods and used it to derive a mechanistic model. Our data identify novel features shared between circadian rhythms and YROs, explain the sequential occurrence of distinct metabolic processes and show how the cellular network architecture is likely to have evolved in response to nutrient limitation.

85 Metabolite Biosensors: Valuable Synthetic Devices for Metabolic Engineering Applications

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Industrially relevant products, including chemicals, fuels and pharmaceuticals, have been successfully produced the last decades by engineering microorganisms, especially the yeast Saccharomyces cerevisiae. Implementation of tools from synthetic biology has greatly improved and accelerated the construction of successful cell factories over the years. One such tool are metabolite biosensors, which have two major applications in cell factory development. One application is to use biosensors for high-throughput screening purposes. This is of great value when screening large-scale libraries, including genomic libraries or libraries created from evolutionary engineering approaches. In principle, a biosensor is developed to sense a compound of interest and couple its production to a phenotypic signal, e.g. fluorescence signal, which allows for high-throughput screening. Another approach, which is more complex but has been shown to be efficient and promising, is to dynamically regulate metabolic pathways in order to decouple the growth phase from the production phase, reduce metabolic burden and the accumulation of potential toxic intermediates.

We are interested in developing and engineering biosensors for S. cerevisiae, specifically biosensors targeting fatty acids and precursors within the fatty acid metabolism as industrially relevant products derived from these are highly attractive. Therefore, we have constructed a fatty acyl-CoA biosensor based on the prokaryotic transcription factor FadR and combined it with an overexpression library to screen for candidate genes that boost the acyl-CoA pool. We evaluated the enriched genes by measuring the fatty alcohol levels, and we found promising targets that are not known to be directly involved in the fatty acid metabolism. We are further interested in expanding the dynamic range of biosensors and engineer promoters with different strengths that are suitable for biosensor applications or in genetic circuit designs. These strategies will be presented at the conference.

86 Understanding Transcriptional Regulation Of Amino Acid Metabolism Through Mapping Of Transcription Factor Binding Sites Using ChIP-exo.

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Amino acids are one of the major building blocks in the cell and are necessary to produce the required proteins and enzymes for cellular growth. To ensure sufficient and balanced amino acid biosynthesis, the expression of enzymes involved in their biosynthesis is heavily influenced by transcriptional regulatory events.

Using the recently developed ChIP-exo method one can identify the binding sites and strengths of a transcription factor (TF) with a higher resolution and less background than previously possible. In this project, we therefore set out to further investigate the transcriptional regulation of amino acid metabolism through mapping the key TFs involved. We are especially interested in how the different TFs respond to a diverse set of environments. To asses this, we performed our experiments using chemostat cultures with fixed growth rates using four different media compositions: two distinct respiratory conditions (aerobic growth on glucose and aerobic growth on ethanol) as well as in two different fermentative conditions (anaerobic growth on glucose and aerobic fermentation using nitrogen limitation).

We started with mapping Gcn4, one of the key regulators in amino acid metabolism. Our data show that Gcn4 has the highest binding activity during aerobic growth on glucose targeting 1388 genes including 80% of all genes associated with the GO term cellular amino acid metabolism. Growth on ethanol as well as anaerobic growth also shows relatively high levels of activity while during growth under nitrogen-limitation only few binding events are observed.

87 Genetic Alterations Mediating Butanol Tolerance in Saccharomyces cerevisiae

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Butanol isoforms are interesting candidates for biotechnical production, both as biofuels and as bulk chemicals. From a fuel perspective, butanol has superior properties vs. ethanol. We and others have published strategies for the production in Saccharomyces cerevisiae. In our case, we have proposed the production of 2-butanol from 2,3-butanediol, via a diol-dehydratase and a secondary alcohol dehydrogenase.

Due to the higher toxicity of butanol vs. ethanol, it is essential to increase tolerance to butanol. A butanol tolerant mutant was obtained from a wild type Saccharomyces cerevisiae strain (JBA) using adaptive laboratory evolution. Proteomics analysis of the resulting mutant strain indicated a rather minor response, mostly manifested in slight upregulation of mitochondrial functions and glycerol production. In order to identify the underlying mutations, we performed whole genome sequencing of the wild type and mutant strains. Surprisingly, this comparison revealed a major shift from a mostly heterozygous tetraploid wild type, to a mostly heterozygous triploid mutant. The wild type differed from the reference genome by approximately 90000 heterozygous SNV's (hzSNV), somewhat fewer in the mutant. No mutations in the mitochondrial genome were identified comparing the two strains. 1800 hzSNV's were unique to the mutant and were randomly distributed. 7300 hzSNV's were unique to the wild type, with allele frequencies agreeing mostly with a loss of ploidy as the cause. However, two different regions on the left arm of chromosome XIV displayed allele frequencies which can not be explained solely by loss of ploidy, indicating an active selection, marking them as especially interesting. One of these regions include the pleiotropic MKT1-locus, allelic variations of which has previously been implicated in stress tolerance. Future work on this project will focus on the possible role of these regions in mediating butanol tolerance.

88 Metabolic Engineering and Adaptive Evolution of Yarrowia Lipolytica for Enabling Effective Xylose Utilization

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Essential for the economic viability of any process is to reduce costs by maximizing the yield or productivity and lower the costs of the substrates necessary for culture media. Lignocellulose materials are one of the most abundant and inexpensive carbon source in nature. They are made primarily of glucose and xylose, with additional small amount of different sugars like arabinose. Biomasses delivered from agronomical residues are potential renewable feedstock for microbial production of valuable substances.

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. It has an efficient and robust native metabolism to produce high levels of organic acids and neutral lipids. Unfortunately, Y. lipolytica is unable to use xylose as a carbon source. In this work we aimed to examined the impact of overexpression of native genes involved in metabolism of xylose - xylulokinase (YALI0F10923), xylitol dehydrogenase (YALI0E12463), xylose reductase (YALI0D07634), and adaptive changes in genetically modified strain during adaptive laboratory evolution. During microbial ALE, a microorganism is cultivated under clearly defined conditions for prolonged periods of time in shake flasks. The adaptive evolution was carried out by the continuous transfer of cells into the fresh medium containing xylose as the sole carbon source.

In order to metabolize xylose, the host must be able to take up pentose inside the cell through sugar transporters. We investigated the functional roles of potential xylose-specific native transporters. Transporters is critical for enhanced xylose assimilation in yeasts, especially in the presence of a competitive glucose substrate. By screening a comprehensive set of putative pentose-specific transporters, we identified a few candidates that could enhanced xylose assimilation. To evaluate expression of enzymes, we conducted the relative quantification of RNA transcript using RT-PCR.

This work demonstrates that effective metabolic engineering may create biological platform for produce compounds from lignocelluloses.

89 MAPK Signaling Pathways in Yarrowia Lipolytica

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Yarrowia lipolytica is a dimorphic yeast with the high potential for industrial application. It is a target of metabolic engineering aiming for increased productivity of polyols, organic acids, lipids and proteins. At the same time, little is known about many aspects of its physiology, which might become an obstacle for further optimization of production processes.

The MAPK signaling pathways related to dimorphic transition and response to environmental stress remain unknown. The only elements of this network identified so far in Y. lipolytica are Ste11 and ylHog1, however the full understanding of their role in metabolism requires additional research.

In this study we performed a knock-out of number of protein kinases in order to determine their roles in MAPK cascades and interactions with ylHog1 and Ste11. Obtained strains were tested for the mycelium growth, erythritol production, sensitivity to environmental stresses and damage to the cell wall. The results indicate the existence of cross-talk between different MAPK cascades and disclose a new potential targets for metabolic engineering.

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90 The standard KanMX gene knockout cassette strongly affects the expression of neighboring genes at transcriptional and translational levels

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The haploid genome of the budding yeast Saccharomyces cerevisiae contains approximately 6000 genes. Two decades ago, the complete gene knockout library was produced by the Saccharomyces Genome Deletion Project with the goal of assigning functions to genes through phenotypic analyses of the mutants. Each gene was replaced with a KanMX cassette harboring the G418 resistance gene under control of the strong eEF1A promoter. The eEF1A terminator, as well as adjacent vector-derived and artificial tag sequences, were inserted along the gene. Thus, the endogenous genetic loci were substantially modified during this procedure. As yeast has a compact genome with short intergenic regions, introduction of a highly expressed gene module could significantly alter local transcription profiles. However, these alterations have never been quantified.

Here, using ribosome profiling and RNA-Seq data for several S.cerevisiae knockout strains, we analyzed transcriptional and translational perturbations induced by the KanMX cassette within the modified genomic loci. In many cases, we discovered significant alterations in gene expression, including severe impairment of translation. These changes could be attributed to shifted transcriptional start sites or activation of alternative polyadenylation signals. The most dramatic changes were observed when a deleted gene was arranged “head-to-head” with the neighboring gene, where a shift of transcription start site of the latter expanded the 5’ untranslated region (UTR), and the appearance of upstream AUG codons, inhibited translation of its main open reading frame. In the “tail to tail” arrangement, activation of alternative polyadenylation signals in the neighboring gene’s transcript and 3’ UTR shortening were found in many cases. In some cases, the dramatic drop in expression level of the neighboring gene agreed with reported genetic interactions of the deleted gene, which can now be viewed as falsely attributed.

Our observations report on the interactions of the KanMX cassette with neighboring genes and provide an understanding of the molecular mechanisms involved. They also suggest that caution is needed in interpreting the results of deletion screens.

The work was supported by a Russian Federation grant (14.W03.31.0012) and the Ministry of Science and Higher Education of the Russian Federation.

93 Contrasting With Expectations, Sit4 and PP2A Dephosphorylate Transcription Activator Gln3 In Nitrogen-Excess, A Condition Where TorC1 Is Activated.

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The yeast Saccharomyces cerevisiae lives in a boom and bust nutritional environment. Sophisticated regulatory systems have evolved to rapidly cope with these changes while preserving intracellular homeostasis. TorC1, a Ser/Thr kinase complex that is activated by excess nitrogen and inhibited by limiting nitrogen or rapamycin treatment, plays a central role in global nutrient-responsive regulation. Two of TorC1's downstream targets are Gln3 and Gat1, the GATA-family activators, whose localization and function are responsible for Nitrogen Catabolite Repression- (NCR-) sensitive transcription. In nitrogen-rich environments, where TorC1 is activated, Gln3 is cytoplasmic (in a Gln3-Ure2 complex) and NCR-sensitive transcription repressed. In contrast, when TorC1 is inhibited by rapamycin treatment, Gln3 is dephosphorylated, dissociates from Ure2, relocates to the nucleus and NCR-sensitive transcription is derepressed. It is generally accepted that in excess nitrogen, where TorC1 is activated, it binds to and inhibits the Tap42-Sit4 and Tap42-PP2A phosphatase complexes that dephosphorylate Gln3, whereas when TorC1 is inhibited, these complexes are released from TorC1, become active and dephosphorylate Gln3. In this way TorC1 achieves nitrogen-responsive Gln3 control.

Though elegant and convincing, the above scenario fails to account for a paradoxical observation, i.e., Sit4 dephosphorylates Gln3 more in nitrogen excess than in nitrogen limiting conditions. This paradox motivated us to revisit the roles of Sit4 and PP2A in Gln3 regulation. Our observations resolve this paradox and add new dimensions to nitrogen-responsive transcription factor regulation: (i) Contrary to expectation, nuclear Gln3 is more phosphorylated than when it is in the cytoplasm. (ii) In nitrogen excess, where TorC1 is activated, Gln3 cycles out of the nucleus into the cytoplasm and there is dephosphorylated by Sit4 and PP2A. (iii) In both excess and limiting nitrogen, Sit4, PP2A and Ure2 are all required to maintain cytoplasmic Gln3 in this dephosphorylated form.

Therefore, Sit4 and PP2A dephosphorylate Gln3 both when TorC1 is activated and inhibited. This is possible because there are two forms of Sit4 and PP2A, free and complexed with Tap42. Only ~5% of Sit4 and PP2A form a complex with Tap42. Tap42-Sit4 and Tap42-PP2A complex activities are controlled by TorC1, whereas free Sit4 and PP2A are not. We deduce that it is the free Sit4 and PP2A that dephosphorylate Gln3 upon its arrival in the cytoplasm. NIH GM-35642-27

94 Nitrogen Limitation Reveals Large Reserves in the Metabolic and Translational Capacities of Yeast.

Rosemary Yu, Kate Campbell, Rui Pereira, Johan Björkeroth, Qi Qi, Egor Vorontsov, Carina Sihlbom and Jens Nielsen

Chalmers University of Technology,Gothenburg University

Cells maintain reserves in their metabolic and translational capacities as a strategy to quickly respond to changing environments. To date, these reserves have not been systematically quantified. Here we quantify these reserves by stepwise reducing the nitrogen availability in yeast steady-state chemostat cultures at a fixed dilution rate, which imposes severe restrictions on total cellular protein and transcript contents. Combining multi-omics analysis with metabolic modeling, we found that 7 metabolic superpathways maintained >50% metabolic capacity in reserve, with glucose metabolism maintaining >80% reserve capacity. We also found that cells maintain >50% reserve translational capacities for 2,490/3,361 (74%) genes, with disproportionately large reserves dedicated to translating metabolic proteins. Finally, we found that ribosome reserves can contain up to 30% sub-stoichiometric ribosomal proteins, and engagement of reserve translational capacities is associated with selective upregulation of 17 ribosomal proteins. Together, our dataset provides a quantitative link between yeast physiology and cellular economics.

95 Production of Abscisic Acid in Saccharomyces cerevisiae.

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With the recent advances in the field of synthetic biology, engineering the metabolism of microorganisms is now simpler and faster than ever before. Despite the rapid progress, examples for introducing multi-step metabolic pathways in a heterologous host are rather sparse. In this project we present one such example, by integrating the genes of the abscisic acid (ABA) biosynthetic pathway from Botrytis cinerea into the genome of Saccharomyces cerevisiae.

The sesquiterpenoid ABA is a signalling molecule first discovered in plants and extensively studied since the 1960s. It plays a pivotal role in abiotic stress resistance in higher plants but is also involved in regulating seed dormancy and fruit ripening. More recently it was discovered that ABA plays a role in human metabolism as well and could potentially be used as a pharmakon for regulating the immune response or to enhance the secretion of insulin.

Since the biosynthetic pathway of ABA in B. cinerea is not completely elucidated yet, we investigated the effects of some of the ABA pathway genes in more detail. Our results show that four genes are sufficient to produce ABA in S. cerevisiae and we could furthermore confirm one of two contradicting studies about the number of essential genes in the ABA pathway.

To spot targets for future strain improvements we analysed the impact of different strain backgrounds with varying precursor and co-factor supply. The proof-of-concept strain was characterised by monitoring ABA production in different growth phases.

This study represents the first step towards a heterologous ABA cell factory for the affordable and sustainable supply of this sesquiterpenoid with applications in agriculture and medicine.

96 Template-limited Transcription Coordinates Histone Homeostasis with DNA Content.

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Protein homeostasis is tightly coordinated with cell growth and size. In larger cells, transcriptional and translational machineries are more abundant and the production of proteins increases accordingly. While well suited for proteins that need to be maintained at a constant concentration, this mechanism imposes a problem for DNA-binding proteins such as histones, which are required at a constant DNA-to-protein stoichiometry instead.

Here, we use the model organism budding yeast to unravel how cells couple histone production to DNA content rather than protein content. By controlling cell size through tunable expression of the cell-size-regulator Whi5, we find that the transcript concentration of control genes such as ACT1 relative to total or ribosomal RNA stays constant with increasing cell-size. In contrast, the relative concentration of histone mRNA decreases in inverse proportion to cell volume. Our data suggest that this differential regulation originates from a distinct cell-size-dependence of histone transcription rates and demonstrate that the promoters alone are sufficient for this behavior.

We conclude from our findings that histone transcription is limited by the DNA template, which is in contrast to most proteins, for which transcription is limited by the transcriptional machinery. Such template limited transcription not only explains the observed cell-size dependence, but also the differential ploidy dependence of transcription driven by histone and control promoters. Thus, our work identifies a potentially widespread mechanism that allows cells to coordinate the homeostasis of DNA binding proteins to DNA content without the need for complex feedback regulation.

97 A Novel Regulator of Senescence.

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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. Interestingly, although DNA binding is impacted in these mutant alleles, transcription of known Tbf1 targets is largely unaffected. In addition, the 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 has allowed us to further investigate the multiple roles of Tbf1.

98 Phosphate Homeostasis and Cell-to-cell Variation in PHO-gene Expression in Saccharomyces cerevisiae is Controlled through 14-3-3 Protein Bmh1, Spl2 and Non-coding RNA transcription.

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The yeast Saccharomyces cerevisiae is an excellent model organism to study the molecular mechanisms governing tight control of phosphate homeostasis. At low extracellular phosphate or potassium concentrations, genes involved in high-affinity uptake of inorganic phosphate (PHO genes) are highly expressed. We have shown (1) that bimodal heterogenic expression of PHO84 (encoding a high affinity Pi transporter) and of SPL2 (encoding a protein with some similarity to cyclin dependent kinase inhibitors) is controlled through 14-3-3 protein Bmh1. Deletion of BMH1 causes a dramatic decrease in the population of cells expressing GFP from a PHO84-promoter and of cells expressing SPL2-GFP (wild type: 80-90% versus bmh1Δ: 20-30%), whereas deletion of homologous BMH2 does not cause a discernable effect.

Since Spl2-GFP is transferred to the nucleus during Pi starvation, we further explored involvement of Bmh1 and Spl2 in Pi-dependent control of transcription. Bmh1 regulation occurs at least partly upstream of the Pho4 transcription factor. Bimodal expression of PHO84 has been shown to be modulated via antisense transcription, under the control of the Rrp6 exonuclease (2). Accordingly, deletion of RRP6 strongly decreases expression of both PHO84 and SPL2.

We further present and discuss a novel model for bimodal, sense-antisense transcription control of yeast high affinity Pi sensing and uptake, involving both 14-3-3 protein Bmh1 and Spl2.

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99 Abolishing Filamentation in Yarrowia lipolytica for Reliable High Lipid Production.

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Chalmers - University of Technology

Yarrowia lipolytica is an oleaginous yeast which has received increased interest in recent years, mostly due to its high lipid content which makes it a good host for the production of various lipids and lipid derivates. However, a disadvantage of Y. lipolytica is its dimorphic growth. In response to the stress of various kinds (e.g. temperature, pH, mechanical stress, osmotic pressure, carbon and nitrogen source) it frequently initiates the formation of filaments. For large-scale industrial applications, this behaviour is highly unfavourable as different morphologies have different effects on the culture rheology and change in cell properties can risk the success of the cultivation.

To overcome the disadvantage of dimorphism, we compared different gene knock outs previously reported to abolish the filamentation. The most reliable target was found to be MHY1 (a.k.a. YlMsn2). Strains with an MHY1 knock out did not show filamentation or aberrant cell morphology under any tested condition. In S. cerevisiae the two MHY1-homologs MSN2/MSN4 have key overlapping roles in mediating the transcription of stress-induced genes in response to a wide variety of adverse conditions, and the double msn2/4-deletion is highly sensitive to stress. Therefore, to investigate whether MHY1 has a similar role in Y. lipolytica, we tested the stress tolerance of strains deleted for MHY1. We have evaluated both long term stress (e.g. C and N-starvation) and acute stress (e.g. heat-shock and oxidative stress survival). Additionally, we evaluated the effect of an MHY1-deletion on the lipid composition and final storage lipid accumulation of the cells under the long-term stress conditions.

100 Molecular differentiation and drug susceptibility of Candida sp. isolated from the Skin of Patients with Acne Clinical Protests

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Introduction: Acne is a pathological disorder and a chronic inflammation in the Sebaceous follicles, and one of the most popular dermatology damages that has affected millions of people worldwide. The aim of this study is to identify Candida species from patients with acne and determine was their drugs susceptibility. Material and Methods: In this cross-sectional study of 70 clinical specimens from suspected skin with acne protests were collected by sterile swab and were streaked on Sabouraud Dextrose Agar containing chloramphenicol. The plates were incubated for 48 hours in c˚37. Suspected colonies were studied through microscopic examination and subsequent passage in accordance with Mycology of standard procedures and specify the type of fungal colony color in CHROM agar for the isolation of the yeast. For final approval, Candida Sp. Sequencing Method (ITS2, ITS4regions) was performed, and susceptibility testing was performed to review Candida sp. for drug-resistant isolates based on CLSI method. Findings: Of 70 clinical isolates studied, 11species of Candida including C.parapsilosis8 (72.73%), C.krusei 1(12.5%), C.lusitaniae 1(12.5%), C.kefir1(12.5%), and a Trichosporon asahi were identified and isolated. C. parapsilosis isolates susceptibility to various concentrations of the anti-fungal agents to isolate Cp1 study has shown that the isolated Cp8 Cp5 with MIC 50 equal to32,0.5,0.25 and MIC90 of <64, <1, <0.5 µg/ml Fluconazole, Itraconazole and Ketoconazole were respectively resistant. Apart from the isolation of Cp1 and Cp8, which had relative strength, almost all other species of C. parapsilosis isolates were susceptible to these drugs Discussion& Conclusion: As various studies have proven that in most cases bacterial agents are involved in causing acne, according to the results of this study, it can be suggested that the yeast Candida sp can be introduced as an agent in the etiology of this disease. Candida species isolates can also be resistant to antifungal drugs and this could be one of several causes why sequential treatment of this disease is defeated always.

101 Mechanisms of Intron Mediated Nutrients Sensing and Resistance to Starvation.

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Most eukaryotic cells including yeast exist in non-proliferative or quiescent state. Surviving this state of non-proliferation is essential for the survival of most microbes and is believed to be regulated mostly through protein signaling pathways like the TOR pathway. Surprisingly, we have discovered that introns which are normally considered junk that needs to be removed by splicing play an important role in mediating cell response to nutrients depletion and the function of the TOR pathway. This striking discovery was obtained by deleting each of the 295 single introns from the yeast genome and analyzing the impact of each deletion on cell growth and gene expression (~ 40 000 growth curves and 10 000 expression data points). Strikingly, we found no correlation between this intron deletion effect on growth and the expression of their host genes. Remarkably, mutated alleles of genes that do not produce proteins still completely complement the growth defect of the introns deletion. Therefore, the intron, and not the protein of the host gene, is required for cell growth in stationary phase. Here we will discuss novel mechanisms and pathways by which introns mediate cell resistance to starvation.

102 Induction of Autophagy and Apoptosis Affects the Gene Expression in Ty Elements of Saccharomyces cerevisiae

Sezai Türkel, Ceyda Çolakoğlu, Eren Tanık and Osman Alp Öztop

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Autophagy and apoptosis are two basic cellular processes that are activated under distinct cellular growth conditions. The molecular components of these two events are well defined in S. cerevisiae. Ty elements are retrotransposon of S. cerevisiae that propagates intracellularly with a retroviral-like mechanism. Their replications require reverse transcription of Ty mRNA and formation of viral-like particles within the yeast cells. It is known that the copy numbers of Ty elements are strictly controlled and do not show significant variations among yeast cells. In this study, we have analyzed the effects of apoptosis and autophagy signaling on the transcription of Ty1 and Ty2. We have found that induction of apoptotic processes by acetic acid results in up to 4-fold decrease in the transcription of Ty1 and Ty2. Moreover, activation of macroautophagy by nitrogen starvation also affects Ty1 and Ty2 transcription but at differential level. While transcription of Ty2 decreases about 2-fold in autophagy inducing growth conditions, transcription of Ty1 is not affected by this growth pattern. In addition, we have also tested the effects of Tor1p, Snf1p and Gcn2p kinases in the apoptosis dependent regulation of Ty2 transcription. Our results indicated that Tor1p and Gcn2p function may be essential for apoptosis dependent down-regulation of Ty2 transcription. Replication cycle of Ty elements also requires programmed ribosomal frame shifting at +1 direction on gag-pol coding junction of Ty mRNA during translation elongation stage. Hence, we have tested the effects of autophagy and apoptosis on the ribosomal frame shift efficiency in Ty1. Activation of apoptosis by acetic acid results with 50% decrease in the ribosomal frame-shifting in Ty1. Overall, results of this study suggest that gene expression in yeast Ty elements are differentially regulated by apoptosis and autophagy signaling in yeasts.

103 New Mitochondrial Function Assays Demonstrate Substantial Differences among Yeasts and Uncover Novel Metabolic Energy Pathways

In Iok Kong and Barry Bochner

Biolog Inc.

With powerful tools for genetic manipulation, Saccharomyces cerevisiae and other yeast species are often used to model, study, and understand human disorders. Many human disorders are due to genetic alterations that diminish cellular energy production, making cells vulnerable. Therefore we worked on developing better methods to assay cell bioenergetics in yeast. The mitochondrion is an organelle of particular interest because functional defects can lead to wide-ranging energetics-related human disorders including cancer, ageing, neurological disorders, metabolic disorders, and immunodeficiencies. We have optimized a new assay approach for measuring electron flow pathways in yeast to replace the multistep procedure of removing the cell wall to generate spheroplasts and purifying mitochondria from the spheroplasts. We found that digitonin can effectively permeabilize the plasma membrane and directly enable mitochondrial metabolism assays. Digitonin sequesters membrane sterols and instantaneously creates pores, allowing the mitochondria to be assayed in situ. The pores allow extracellular small molecules to enter the cell while cytoplasmic small molecules leak out. Our assay approach has allowed us to utilize the PM1 MicroPlate™, a panel of 95 biochemical substrates, along with a tetrazolium redox dye mix, to examine both the live cell metabolism using unpermeabilized cells, and the mitochondrial metabolism using permeabilized cells. It is clear that the cellular energy pathways are regulated by the chemical composition of the culture medium as well as other culture parameters such as temperature. The induction of mitochondrial metabolism is strong in cells cultured in (respiratory) lactate plus glycerol-containing media whereas cytoplasmic energy pathways dominate in cells cultured in glucose-containing media. We have explored how mitochondrial metabolism differs in Saccharomyces cerevisiae and four other yeasts: Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, and Candida albicans. We can observe clear metabolic differences even in closely related strains of Saccharomyces cerevisiae. Previously unrecognized energy pathways were also discovered, for example including D-malic acid, D-galactonic acid g-lactone, and glycolic acid.

104 Transcriptional Complexity and Functional Consequences of Cryptic Promoters

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Cryptic transcription is widespread and generates a heterogeneous group of RNA molecules of unknown function. To improve our understanding of cryptic transcription, we investigated their transcription start site usage, chromatin organization and post-transcriptional consequences in Saccharomyces cerevisiae. We show that transcription start sites (TSSs) of chromatin-sensitive internal cryptic transcripts retain comparable features of canonical TSSs in terms of DNA sequence, directionality and chromatin accessibility. We define the 5’ and 3’ boundaries of cryptic transcripts and show that, contrary to RNA degradation-sensitive ones, they often overlap with the end of the gene thereby using the canonical polyadenylation site and associate to polyribosomes. In fact, we show that chromatin-sensitive cryptic transcripts can be recognized by ribosomes and may produce truncated polypeptides from downstream, in-frame start codons. Our work suggests that a fraction of chromatin-sensitive internal cryptic promoters are in fact alternative truncated mRNA isoforms. The expression of these chromatin-sensitive isoforms is conserved from yeast to human expanding the functional consequences of cryptic transcription and proteome complexity.

To further expand our understanding of transcriptome complexity, we will present also our recent work regarding how an optimized version the TIF-Seq approach (TIFSeq2) can be used to interrogate the transcriptional complexity from yeast to human.

105 The posttranscriptional modification of a conserved iron repressor defines an unexpected detoxification role in Candida albicans gastrointestinal commensalism

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Candida albicans is the most common opportunistic fungal pathogen in human, capable of causing mucosal and systemic infections in immunocompromised individuals. To survive in the host, iron acquisition and avoidance of the immune oxidative burst are two critical barriers. Here we reported an unexpected detoxification role of the iron-responsive transcriptional repressor Hap43 under iron replete conditions, as we observed that a high level of ROS, which generated by the Fenton reaction, triggers sequential posttranscriptional modifications of this transcriptional repressor, including phosphorylation, nuclear-to-cytoplasmic translocation and ubiquitination. Importantly, protein modifications result in protein degradation through an ubiquitin-proteasome pathway and ultimately de-repression of its downstream target genes related to anti-oxidation, and thus removing the deleterious effect of the ROS-triggered cytotoxicity. Consistently, a mutant deleting all potential phosphorylation sites of Hap43 accelerates sensitivity of oxidative stress and thus significantly attenuates its survival and colonization in gastrointestinal tract in both Drosophila Melanogaster and mouse iron-overload models. In conclusion, our results define an unexpected regulatory role of the iron responsive transcription factor Hap43 through protein posttranscriptional modifications in promoting gastrointestinal commensalism of C. albicans.

106 The Many Roles of Elg1 in the Maintenance of Genome Stability and Chromatin States

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Tel Aviv University

The Many Roles of Elg1 in the Maintenance of Genome Stability and Chromatin States

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Timely unloading of the DNA polymerase processivity factor PCNA from the replication fork is essential for genome integrity. The yeast Elg1 interacts with the Rfc2-5 subunits of the Replication Factor C (RFC) complex to form an RFC-like complex (RLC). The Elg1-RLC is the principal unloader of chromatin-bound PCNA, and thus plays a central role in DNA replication, DNA recombination and genomic stability maintenance. Mutations in Elg1 lead to hyper-recombination, chromosome loss, longer telomeres, hyper-transposition, chromatin silencing problems and sensitivity to DNA damage agents. Thus, Elg1 affects a number of central processes in the cell. In addition, Elg1 is a target of the DNA damage Response (DDR): when cells are subjected to DNA damage, it becomes phosphorylated at its Serine 112 in a Mec1 (ATR)-dependent fashion. Using a chromatin-fractionation assay, we show that phosphodead elg1 mutants are defective in PCNA unloading upon DNA damage, but are proficient for PCNA unloading during normal S-phase replication. Our results show that, paradoxically, Elg1 plays a role in eliciting the DDR, in addition of being its target. We will discuss possible mechanisms by which Elg1 function links chromatin maintenance and epigenetic memory to DNA replication and DNA repair.

107 Saccharomycopsis Yeasts: Omics Insight Into These Unique Fungal Predators

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Predator yeasts are either homothallic or heterothallic yeasts that belong to the genus Saccharomycopsis. They share the unique trait of being able to kill other fungal cells via forming a penetration peg that promotes digesting and feeding on the cellular contents of the prey cells. We recently showed that Saccharomycopsis schoenii is to be able to kill cells of several Candida species including the emerging multi-drug resistant human fungal pathogen Candida auris. However, the molecular mechanisms involved in the predatory activity of S. schoenii have not been explored. To this end, we de novo sequenced, assembled and annotated a draft genome of S. schoenii. Using proteomics, we confirmed that Saccharomycopsis yeasts have reassigned the CTG codon and translate CTG into serine instead of leucine. Further, we confirmed the absence of all genes of the sulfate assimilation pathway in several Saccharomycopsis species, and detected expansions of several gene families, including flocculation genes, chitinases, glucanases as well as aspartic proteases. The FLO/ALS-like Sequence (FAS)-gene family of S. fermentans shows similar telomere expansion as in Saccharomyces cerevisiae. Furthermore, flocculation at the end of fermentation in S. fermentans is also Ca2+-dependent. Using Saccharomyces cerevisiae as a model prey cell, we analyzed the timing and nutritional conditions under which S. schoenii kills prey cells. We found that nutrition limitation, particularly methionine deprivation, can trigger predatory activity. Upregulation of genes seen under methionine deprivation were alleviated when S. cerevisiae was available as prey, which suggests that feeding on prey cells can supply the necessary nutrient and alleviate hunger stress. During predation, both proteomic and transcriptomic analyses revealed that S. schoenii highly upregulated and translated aspartic protease genes, probably used to break down prey cell walls or fungal proteins. We have now embarked on gene-function analyses to identify key regulators of this predation behavior in Saccharomycopsis. Current advances will be presented including our first attempts on complete ORF deletions in S. schoenii

108 Production of Branched Fatty Acids in Yarrowia lipolytica.

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Up to 20 billion liters of lubricant enter the environment each year via routine use, spills, and disposal, often necessitating expensive remediation. Biobased and biodegradable lubricants currently account for less than 2% of the global lubricant supply due to inferior qualities. To provide renewable alternatives, we have used Yarrowia lipolytica to produce 10-methyl branched fatty acids (BFAs). We cloned and characterized a two-step bfa production pathway, in which bfaB methylates delta9 unsaturated fatty acids to form 10-methylene BFAs. Subsequently, bfaA reduces the double bond to produce a fully saturated 10-methyl BFA. Expression of bfaB in yeast enabled abundant production of C16 and C18 10-methylene BFAs. Overproduction of a fusion protein, bfaA-B, led to efficient accumulation of 10-methyl palmitate and 10-methyl stearate. This yeast system provides a feasible, biobased alternative to produce branched fatty acids suitable for industrial and consumer markets.

109 Different Strength Feedback Loops a Mechanism Behind Active Paralogous Compensation

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Gene duplicates provide robustness against genetic perturbations, when a member of a pair is deleted the remaining one can fulfill the function of the deleted copy, maintaining the system operational. In some cases transcriptional reprograming occurs on the remaining pair, a so called active paralogous compensation. To understand this cellular responses, we used the Leucine and Valine metabolic pathway as a case study, in which two pairs of duplicated genes LEU4/LEU9 and BAT1/BAT2 are involved. Both paralogous copies participate in at least one of the negative feedback loops of the pathway. We followed the changes on protein abundance of all the viable knockout mutants of the system at the single-cell level using high-throughput flow cytometry. We found that while the leu4Δ and bat1Δ mutants reprogram the overall proteomic profile of the pathway, the remaining mutants have a minor or no effect on the enzyme levels of the system. Such responses places Leu4 and Bat1 feedback loops as the major regulators of the Leu/Val pathway. Notably, the active response of the remaining Leu9 and Bat1 upon the deletion of their cognate copies (leu4Δ and bat1Δ, respectively) allows them to substitute the catalytic activity of their pairs, but changes the strength of their respective feedback loops, preventing Leu9 and Bat1 to compensate the regulatory effect exerted by their pairs over the pathway. Here we introduce the change in the strength of the feedback loops as a mechanism behind the active paralogous compensation and show how paralogous active transcriptional reprogramming is not exclusive of the paralogous pairs but extents to the whole metabolic pathway. Further, we developed a computational model to analyze the benefit of having a system with two alternative feedback strengths, such as two isozymes with different regulatory properties. We observed that a system with a two isozyme system can produce different strength feedback loops generating a variety of output concentrations with the same amount of enzyme and can respond to a wider input concentration range than a system with only one enzyme. Together, the experimental evidence and the theoretical approach used in this work support that duplicated genes have been retain due the acquisition of new regulatory capabilities and reveals that although paralogous pairs have a redundant catalytic activity they have a non overlapping regulatory functions.

110 Mapping the interaction network of ubiquitylation enzymes in living cells

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Ubiquitylation is one of the most sophisticated and versatile post-translational protein modifications. It is achieved through the concerted action of ubiquitin conjugating and ligating enzymes (E2s and E3s) that transiently interact with each other as well as with their modification substrates. These enzymes constitute a dynamic network that comprises more than 30 E2s and 600 E3s in human cells. Although the molecular determinants of E2-E3 interactions have been investigated for a few prototypical E2-E3 pairs, it is currently not possible to predict which E2s and E3s function together and most physiological E2-E3 pairs remain to be identified.

To further characterize the cellular network of E2 and E3 enzymes we aimed to directly probe E2-E3 interactions in living cells. Using budding yeast as a model organism, we found that a protein complementation assay based on the NanoLuc luciferase enables robust detection of well-described E2-E3 pairs expressed in near endogenous conditions. For instance, we readily detected the interaction between Ubc13 and Rad5, the E2 and E3 that are responsible for the polyubiquitylation of the replication processivity factor PCNA. The assay is specific since the interaction signal is strongly reduced in cells expressing Ubc13 mutated in its E3 interaction surface.

We used this assay to systematically map E2-E3 interactions in living yeast cells. Our results show that the E2-E3 network is surprisingly sparse, with most E3s interacting primarily with a single or a few E2s. Moreover the network reveals striking discrepancies in E2 connectivity. Some E2s, like Rad6, Cdc34 or Ubc7, appear to be highly specialized and to interact with a limited set of E3s. In contrast, other E2s, like Ubc4, are generic and interact with numerous E3s. These findings and their implications for ubiquitin signaling will be discussed.

111 In Depth Physiological Characterization Reveals a Novel Survival Strategy for the Yeast Debaryomyces hansenii at Very High Salinity, Confirming its Halophilic Behavior.

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Debaryomyces hansenii is traditionally described as a halotolerant non-conventional yeast, being the model organism for the study of osmo- and salt tolerance mechanisms in eukaryotic systems, for the past 30 years (Adler et al., 1985; Prista et al., 1997, 2005). Its halotolerant nature has been confirmed by the fact that the presence of sodium in the culture media protects the yeast cells against oxidative stress and additional abiotic stresses, like extreme pH or high temperature (Almagro et al., 2000; Papouskova and Sychrova, 2007; Navarrete et al., 2009).

However, the study of D. hansenii´s biotechnological potential has always been difficult due to the persistent limitations in the availability of highly efficient molecular tools described for this yeast. There is also a lack of consensus and contradictory information along the recent years that limits the fully understanding of its carbon metabolism and physiological characterization in controlled and monitored environments. Moreover, there is also controversy about the diversity in the culture conditions (media composition, temperature and pH among others) used by different groups, which makes it complicated when trying to get conclusions and behavioral patterns.

In this work we present for the first time a complete physiological characterization of D. hansenii during batch cultivations, by using highly instrumented and controlled bioreactors at lab-scale. Our findings show a more complete picture of the central carbon metabolism, and the external pH influence on the yeast capacity to tolerate high Na+ and K+ concentrations are also presented. Finally, the controversial halophilic/halotolerant character of this yeast is further discussed and a novel survival strategy and adaptative behavior to high saline environments suggested.

112 Correlation Of MAPK Activity And Gene Expression In Single Cells

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In higher eukaryotes, Mitogen-Activated Protein Kinases (MAPK) control the induction of complex transcriptional programs. These newly transcribed proteins will in turn play a key role in multiple cellular processes including transient adaptation to stresses and cell-fate decisions. Misregulation of MAPK gene expression has been linked to numerous diseases. It is therefore important to understand how the MAPKs control their downstream transcriptional targets. In order to achieve this, we use the HOG MAPK cascade in budding yeast as a prototypical stress-response pathway. We developed fluorescent biosensors to monitor in single cells the dynamics of kinase activity by measuring the relocation of the MAPK Hog1. The induction of transcription was quantified using the PP7 system by following the production of mRNA at the transcription site. In addition, we developed a dynamic expression reporter system to monitor the production of protein from a promoter of interest in real time. The activation of multiple stress-response promoters upon mild osmotic shock was compared, allowing to precisely quantify the timing of gene expression. This comparison allowed to observe which parameters are dependent on Hog1 activity and which ones are controlled directly by the promoter identity. In addition, we perturbed the nuclear relocation of the MAPK, in order to observe how the enrichment of the MAPK in the nucleus contributes to the efficient activation of stress responsive genes.

113 Activity Of Yeast Nutrient Signalling Pathways During Wine Fermentation.

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The yeast Saccharomyces cerevisiae is the main microorganism carrying out the alcoholic fermentation to convert grape juice to wine. The grape juice is characterized by plenty sugar concentration, in the form of glucose and fructose, that causes hyperosmotic stress, but scarce nitrogen sources, so nitrogen limitation usually produces starvation during most fermentations. These peculiar conditions make the crosstalk between nutrients signalling pathways a critical event not only for cell survival, but also for proper fermentation performance in metabolically active cells. As nitrogen is scarce, the TORC1 pathway must be relevant in the process. TORC1 activity was measured by the phosphorylation state of two of its readouts, the proteins Rps6p and Par32p. The analysis of both TORC1 targets indicates that their branches pathway are activated only during the first hours of winemaking pointing out that signalling of nitrogen shortage may be taking place earlier than expected, when nitrogen is still available. Possibly, other nutrient signalling pathways could be active to promote growth. One of these pathways is the glucose repression pathway, controlled by protein kinase Snf1p, mainly regulating the use of carbon sources alternative to glucose. Snf1p-dependent phosphorylation patterns demonstrate that surprisingly this pathway is also active only during the first hours of winemaking, when glucose and fructose are present at high concentration much longer. The activity of the Ras/cAMp/PKA pathway has also been investigated by measuring cAMP levels, which show a 2.5-fold reduction between the first hours of fermentation and stationary phase cultures, when the pathway is not activated. Stress conditions at the beginning of fermentation may give rise to a certain level of kinase activation, despite the high abundance of sugars in the must.

Nitrogen availability also influences the activity of yeast nitrogen catabolic repression (NCR), mediated by the transcription factor Gln3p, which allows cells to repress the use of poor nitrogen sources when optimal nitrogen sources are present. The analysis of Gln3p phosphorylation state along fermentation indicates that NCR seems to be inactive at the beginning of the fermentation, although yeast assimilable nitrogen (YAN) is not low.

The coordination between nutrient signalling pathways allows to integrate different inputs, such as nitrogen shortage and high sugar concentration, to respond to the specifically environmental conditions during the alcoholic fermentation of the grape must, which are very harsh.

115 Erv14 Cargo Receptor of COPII Vesicles Is Required for Trafficking of Membrane Proteins with Large Hydrophilic Moieties

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Erv14 is required for an efficient targeting of more than 30 proteins to the yeast plasma membrane. A joint structural feature, for which they need the help of Erv14, is not known. ERV14 deletion renders S. cerevisiae cells sensitive to both low and high concentrations of alkali metal cations suggesting its role in the trafficking of monovalent cation importers and exporters. We have tested all plasma-membrane monovalent cation transporters and identified three of them (Trk1, potassium-specific importer; Tok1, potassium-specific outward rectifying channel; Nha1, potassium and sodium exporter exchanging cations for protons) as requiring the presence of Erv14. The three proteins are long polytopic membrane proteins with a large cytoplasmically oriented part, for example, a very long hydrophilic C-terminus of Nha1 or a very large loop between 2nd and 3rd transmembrane segment of Trk1. In contrast to the full version, truncated Nha1, lacking almost complete C-terminus, still binds to Erv14 but does not need it to be targeted to the plasma membrane. This result suggested that Erv14 is important for the delivery of plasma-membrane proteins with large hydrophilic parts. To test this hypothesis, we used three sets of experiments. We prepared a series of ScNha1 proteins with different size of their C-termini, and tested their cell localization and activity in the presence or absence of Erv14. Similarly, we tested the dependence of heterologous yeast Nha1 antiporters (differing in the length of their C-termini) on the presence of Erv14 in S. cerevisiae cells. Finally, we prepared Trk1 versions with truncated intracellular loop, and again tested their activity and cell localization in the presence or absence of Erv14. For all tested proteins, a shorter hydrophilic part resulted in their better delivery to the plasma membrane in erv14Δ cells. Obtained results suggest that Erv14 is indispensable for a smooth delivery of membrane proteins with very long hydrophilic parts to the plasma membrane.

116 Start is not the Metabolic Commitment Point of the Yeast Cell Cycle.

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Cells have complex regulatory mechanisms to control and coordinate their metabolism, growth and division. When proper regulation is disturbed, cells can develop disease states like cancer. In this project, we use the model organism budding yeast to study how cell division is coordinated with nutrient supply. The current text-book knowledge states that metabolic signalling mainly affects the cell cycle up until the cell cycle commitment point termed Start. Start is defined as the time when 50% of the transcriptional inhibitor Whi5 exits the nucleus. However, we hypothesize that to properly coordinate metabolism, growth, and division, single cells should exhibit a cell-cycle dependent response to metabolic perturbations even after Start and any time during growth and division.

To test the effect of metabolic perturbations on cell cycle progression in single cells, we imposed glucose switches while imaging fluorescently labeled cell cycle markers in live cells. We found that 89.5% of the post-Start cells stopped or delayed their cell cycle when exposed to glucose starvation. Surprisingly, we found that cells that had just passed the cell cycle commitment point Start could transport Whi5 back from the cytoplasm into the nucleus and re-enter a pre-Start state. This phenomenon was as high as 90% for cells exposed to starvation within the first 30 minutes after they passed Start. This clearly indicates that Start is not the ultimate point of commitment and irreversibility vis a vis metabolic perturbations. As has been suggested for mammalian cells, there may be different points of commitment in G1 for hormones and growth factors one the one hand, and metabolic signals on the other hand. In the light of our findings, we suggest that there are unrevealed checkpoints that coordinate cell cycle entry and progression with metabolic signals.

117 14-3-3 Proteins Regulates Transport Activity of Saccharomyces cerevisiae Na+, K+/H+ Antiporter Nha1 by Binding to Serine 481

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An appropriate concentration of monovalent cations (Na+, K+ and H+) is crucial for the majority of cell functions. Na+/H+ antiporters belong among the cation transport systems that participate in ensuring the optimal intracellular level of alkali-metal cations and protons in cells of most organisms. In S. cerevisiae, the plasma-membrane Na+, K+/H+ antiporter Nha1 is a housekeeping protein that together with the main cation efflux systems (Ena ATPases) enables the growth of cells in the presence of high concentrations of alkali-metal-cations salts. Due to its ability to export K+ cations, Nha1 also plays an important role in the regulation of internal pH and membrane potential, in immediate cell response to osmotic stress or in the regulation of cell cycle. The presence of Nha1 long C-terminus (554 amino acid residues, i.e. 56.2% of the whole 985-aa long protein) is important for all these processes. The Nha1 was previously shown to interact with the yeast 14-3-3 isoform (Bmh2), but neither the site nor the importance of the binding were identified. In this work, we searched for Nha1 residues involved in the interaction with 14-3-3 proteins. Tests of interaction in vitro between the recombinantly prepared Nha1440-596 C-terminal polypeptide and Bmh proteins showed that phosphorylated Ser481 is the 14-3-3 binding site and the resolved crystal structure of Bmh2 with the phosphopeptide containing pSer481 confirmed it. In addition, our structural analyses indicate that the C-terminal part of Nha1 is disordered and the 14-3-3 binding induces its disorder-to-order transition. Set of experiments in vivo showed that the lack of putative phosphorylation site at Ser481 in the 14-3-3 binding motif significantly increases the cation efflux activity via Nha1. Taken together, the binding of 14-3-3 seems to be essential for the negative regulation of Nha1 activity, which should be low under standard growth conditions, when low amounts of toxic (Na+ and Li+) salts are present, and the yeast cells need to accumulate high amounts of K+.

118 Multiple Layers of Phospho-Regulation Coordinate Metabolism and the Cell Cycle.

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The coordination of metabolism, growth and cell division is crucial to maintain a healthy cell. During the cell division cycle in budding yeast we previously showed that over half of the metabolome changes in concentration, suggesting that metabolic fluxes are adjusted to support different cell cycle phases. However, how these adjustments are achieved remains largely unknown. Since the cell cycle is regulated to a large extend by protein phosphorylation, we set out to investigate the phospho-regulation of metabolism during cell cycle progression.

We measured the phosphoproteome across ten time points during the cell cycle of yeast growing on ethanol. Using a tandem-mass-tagging approach, we quantified the time profiles of over 11 000 phosphorylation sites on more than 2000 proteins. We found over 110 sites on metabolic enzymes and transporters to be regulated. These were distributed among many pathways including carbohydrate catabolism, lipid metabolism and amino acid synthesis.

Among all increasing phosphorylation sites an unbiased motif analysis yielded strong enrichment of the CDK consensus motif, but also a second, arginine-directed motif. This points to an involvement of the protein-kinase A, known to sense nutrients and to regulate carbon metabolism and growth. We provide evidence that the PKA pathway itself is regulated by the cell cycle. Notably, we also find over one thousand sites that are dephosphorylated during the early cell cycle, including many sites on metabolic proteins. The phosphatase Glc7, known to regulate both the cell cycle and carbon metabolism, may play an important role. Its regulatory subunits such as Reg1 and Gac1 appear to be phospho-regulated, which may direct Glc7 towards metabolic targets during the cell cycle.

In summary, our results tighten the link between metabolism and cell cycle progression, and provide evidence for multiple layers of cross-talk.

119 The Telomeric DNA Terminal 5' end Sequence is Highly Regulated in the Budding Yeast Naumovozyma castellii.

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Lund University

Telomeres are specialized chromatin structures located at the end of linear chromosomes, that form protective cap structures to maintain chromosomal integrity. The telomeric DNA sequences are composed of short tandem repeats and are bound by an array of proteins. In the budding yeast Naumovozyma castellii, Rap1 binds to telomeric double-stranded (ds) DNA and Cdc13 binds to the single-stranded (ss) 3' overhang, and recruit several other proteins to the telomere. The junction between the ds and ss telomeric DNA, the ds-ss junction, is fundamental in the protection and maintenance of the telomeric structure, as it dictates the

interactions between Rap1 and Cdc13. To investigate the ds-ss junction in N. castellii, we aimed to determine whether there is a preference for a specific 5' end terminal nucleotide within its 8 bp telomeric repeat. To this end, we developed a PCR-based method, termed Permutation-Specific Telomere PCR (PST-PCR). Out of the eight different possible permutations, we observed a prominent preference for three consecutive 5’ end bases in the wild-type strain of N. castellii. Observations of the same permutation preference in other strains of N. castellii indicate that this is a conserved feature. Moreover, PST-PCR analyses of cells at different growth stages showed that the permutation preference is stably kept at different phases of the growth curve. Strikingly, some permutations are completely absent at the 5' end, suggesting that they are avoided by the cell. Thus, our results indicate that the telomeric 5' ends are highly regulated to encompass distinct permutations of the telomeric sequence, implying that a specific ds-ss junction structure may be necessary for the establishment of the protective telomere cap structure.

120 Rewiring of Saccharomyces Cerevisiae Metabolism for Heme Production Using Genome-scale Modeling.

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Heme is a cofactor of several enzymes of industrial interest and improving its intracellular production is attractive for the development of strains capable of high synthesis of heme proteins. In this study, we applied Saccharomyces cerevisiae genome-scale metabolic model (GEM) to deduce fluxes and genes important for the improvement of heme production from glucose. The in silico simulations of different heme and biomass yields highlighted the importance of several metabolic fluxes in balancing biomass for heme production. The simulations returned 84 genes target hits, associated to reactions that showed flux changes during different simulation conditions. The deduced genes were further analyzed by the impact of their deletion and overexpression on heme biosynthesis. The individual experimental modifications of gene targets expression resulted in up to 300% increase in heme production from glucose in engineered strains. In addition to genes of heme biosynthesis, genes involved in glycolysis, pyruvate, Fe-S clusters, glycine and succinyl-CoA metabolism were found important for the overproduction of heme.

121 A Novel Method to Follow RNA Expression in Live Yeast Cells at the Single-Cell Level Over the Cell Cycle and Multiple Generations

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Correct regulation of transcription and inheritance of transcriptional programs through cellular divisions is important for the survival of all organisms. Despite the fundamental role RNA molecules play in controlling cell functions, experimental approaches enabling the observation of transcription of particular RNA species in live cells are still very limited. Currently, expression levels of mRNAs can be studied indirectly by means of fluorescent tagging of the encoded proteins, and fluorescent reporter proteins can be used to study ncRNAs. A major limitation of these approaches is that they only provide indirect information at the protein level rather than RNA transcription itself.

To overcome this limitation, we combined a recently developed RNA aptamer iSpinach method (A. Autour et al. Nucl Acid Res, 2016) with a dedicated microfluidics based approach for single live-cell imaging to follow mRNA and ncRNA levels with high time resolution over multiple generations in budding yeast. Briefly, iSpinach binds the 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI) fluorophore or its derivative, resulting in a fluorescent signal. To validate our method, we tested a large variety of RNAs, including mRNAs and ncRNAs expressed from high and low copy number plasmids, plasmids integrated into the yeast genome, as well as endogenous tagging of mRNAs and ncRNAs. Developing several image-analysis approaches for unbiased analysis of RNA expression levels we carefully characterized the potential of this method. We find that in combination with our single-cell microfluidics approach and our image analysis pipeline, RNA aptamer iSpinach-labelling enables direct visualization of gene expression strength, cell cycle dynamics, and expression pattern inheritance at the single cell level.

In summary, we established a method which allows studying RNA transcription directly, over a long period of time and at the single-cell level.

122 Alternative Lengthening of Telomeres in the Budding Yeast Naumovozyma castellii.

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Telomeres are specialized chromatin structures at the ends of eukaryotic chromosomes. They provide genome stability by protecting from exonucleolytic degradation and assures that natural DNA ends avoid detection by DNA damage response pathways and end-to-end fusion events. The enzyme telomerase is crucial for the extension and maintenance of the telomeric DNA, since the conventional replication machinery cannot copy linear genomic DNA molecules to the terminus. Telomerase extends the telomeric single-stranded 3' overhangs by adding short DNA tandem repeats. The budding yeast N. castellii shows a prominent and processive telomerase activity in vitro, adding several 8 nt repeats to telomeric 3' ends. Remarkably, in spite of its prominent telomerase activity, N. castellii showed high proficiency to survive when telomerase was inactivated. Disruption of the TLC1 gene, encoding the telomerase RNA component, generated survivors at high frequencies. These survivors maintain their telomeres by alternative mechanisms; called Alternative Lengthening of Telomeres (ALT). Both haploid and diploid strains were passaged for >300 generations without exhibiting any major change in colony morphology. In contrast to S. cerevisiae, ALT survivors of N. castellii don't exhibit any major growth crisis at any time-point during the passaging, although a slight variation in growth rate and colony size was observed. Pulsed Field Gel Electrophoresis revealed that survivors sustain linear chromosomes. However, analysis of the organization of the chromosome termini showed that telomeres experience rapid telomere attrition in the early passages, while they undergo incremental lengthening in later passages. Disruption of the EST1 gene, encoding a protein sub-component of the telomerase holoenzyme, generated mutants with a similar survival capacity, sharing the same telomere lengthening phenomenon. To elucidate the molecular mechanism of the ALT pathway, we isolated the terminal DNA sequences and revealed the sequences forming the functional caps of the knockout strain chromosomes. We conclude that the ALT pathway is very quickly and efficiently activated when telomerase is disrupted in N. castellii. The observed incremental lengthening of telomeres is a general rescue mechanism used by N. castellii and we propose a model for the molecular mechanism of this specific ALT pathway.

123 Assessing the Potential of Micro-fermentations through Screening and Media Optimisation of Recombinant β-carotene Production by the Yeast Yarrowia lipolytica

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The demand for β-carotene as a natural pigment and an antioxidant has increased in previous years. All projections show that the demand will increase even more in the coming years [1], [2]. Currently β-carotene is produced either chemically or synthetically, the latter has been linked to multiple negative effects including cancer [3]–[7]. These concerns, in addition to a more critical view on synthetic colorants from the public has offset the search for natural or recombinant production platforms. Low productivities and difficulties are some of the issues associated to the few existing naturally-derived production systems [4], [6], [8].

As a result, we chose to focus on the production of recombinant β-carotene from an engineered Yarrowia lipolytica strain [1], and how we could optimise the production capacity as much as possible through media optimisation. In Yarrowia, the terpenoid pathway and lipogenesis share a common precursor, therefore we hypothesised that optimising the lipogenesis would also optimise the synthesis of β-carotene. To maximise the number of parameters in our screening we used the BioLector I micro-fermentation system, which allowed us to run 48 micro-fermentations simultaneously.

Among our findings, we identified parameters to optimise in order to target specific points in the metabolism and determined their optimum values. Through scale-up of experiments from micro-scale to benchtop laboratory-scale bioreactors we determined the scalability of the BioLector system to be accurate. We thereby proved that the BioLector I can be used in studies like ours and in studies were a fast and accurate screening method in needed.

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124 The Interplay of Multiple Phosphorylation Sites Regulates the Trehalase Nth1.

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The adjustment of cellular activities must occur on a very short time scale when the environment changes. For achieving this, proteins are often post-translationally modified by phosphorylation. These modifications often occur not only at one but at multiple sites. Multi-site phosphorylations can enable the adjustment of its own activity, docking with other proteins and act as signal integrators. However, the hierarchy and interplay within multi-site phosphorylations for these regulatory functions are largely unknown.

Here we study multi-site phosphorylation using the trehalase Nth1 as a model. Nth1 is known to be activated by the metabolic kinase PKA by phosphorylation of four serine residues S20, S21, S60 and S83, and by the kinase CDK on S66. However, how these different sites interact and how they contribute to Nth1 activation remains largely unknown. Here, we use different biochemical approaches, including phos-tag-SDS-polyacrylamide gels and trehalase assays, to investigate the in vivo function and relevance of the four PKA sites.

We show that there is a hierarchy within the PKA phospho-sites: In contrast to S20 and S83, which are constitutively phosphorylated, the weaker PKA sites S21 and S60 are only phosphorylated by an increase of PKA activity. The simultaneous phosphorylation of the two sites S60 and S83 is required for the binding of the dimeric activator protein Bmh1. The constitutively phosphorylated site S83 thereby probably function as the high-affinity “gatekeeper site” possibly able to bind to one monomeric subunit. However, the successful binding of the Bmh1 dimer and thus Nth1 activation depends on the weaker PKA site S60 as secondary, low-affinity site and is thus a measure for PKA activity. However, full trehalase activity additionally depends on the phospho-sites S20 and S21. Because the phosphorylation of these two sites occurs prior S60, it seems likely that S20/21 support the phosphorylation of the other PKA sites.

Under slow-growth conditions with low basal PKA activity, the cell cycle dependent phosphorylation of S66 by CDK contribute to Nth1 activation, maybe by supporting Bmh1 binding. This interplay and hierarchy within the individual phospho-sites enables the rapid activation while recovering from the stationary phase as well as the fine-tuning for the oscillating trehalase activity over the cell cycle under slow-growth conditions.

125 Subcelullar Localization and Leucine Feedback Control of Equivalent Paralogous Saccharomyces cerevisiae and Kluyveromyces lactis Proteins Play Key Functional Roles in Subfunctionalization.

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Analysis of the mechanisms involved in subfunctionalization of S. cerevisiae ScLEU4/ScLEU9 paralogous genes pertaining the leucine biosynthetic pathway, encoding □-isopropylmalate synthases (□-IPMS), showed that ScLeu4-4, ScLeu9-9 homodimers and an ScLeu4-ScLeu9 heterodimer can be formed, each isozyme displaying differential leucine sensitivity and thus distinct feedback control of the leucine biosynthetic pathway (IC50: ScLeu4-Leu4-0.03, ScLeu9-Leu9-1.10 and ScLeu4-Leu9-0.16). ScLeu4 and ScLeu9 monomers are both mitochondrially localized allowing homo and heterodimer formation. Although the lineage which gave rise to Kluyveromyces lactis (K. lactis) did not underwent a process of (WGD), it has been found that this aerobic yeast has retained duplicated gene pairs such as KlLEU4 and KlLEU4BIS, generated by sporadic duplications.

We will present results showing that as opposed to that found for the S. cerevisiae paralogous pair, KlLEU4 and KlLEU4BIS diversification has resulted in differential localization of encoded enzymes, such that the monomer encoded by the ScLEU4/ScLEU9 syntenic KlLEU4 gene is located in the cytosol, while the one encoded by the non-syntenic KlLEU4BIS is mitochondrial. Accordingly KlLeu4 mitochondrial non synthenic isozyme complements an Scleu4□ mutant which recovers wild type phenotype. A syntenic KlLeu enzyme harboring a mitochondrial localization signal is being constructed, in order to determine whether it can confer wild type phenotype to the Scleu4□/KlLEU4-mit mutant. In regard to leucine sensitivity, both, syntenic and non-syntenic isozymes show similar leucine reactivity (KlLeu4 IC50 0.03 and KlLeuBIS IC50 0.020). Lack of either KlLeu4 or KlLeu4BIS does not result in decreased growth of single mutants and only the double Klleu4□ Klleu4BIS□ mutant displays leucine auxotrophy, when grown on either glucose or ethanol as sole carbon sources, leucine addition or ScLEU4 transformation restores WT growth. It can be concluded that ScLEU4 and KlLEU4BIS are orthologous genes and that the subcellular localization and leucine sensitivity of their encoded products play a crucial role in functional diversification. The fact that the growth phenotype of a Klleu4□ Klleu4BIS□ is fully reverted to wild type through leucine addition shows that □-IPMS localization plays no functional additional role. The functional role of ScLeu4 and ScLeu9 mitochondrial localization will be discussed.

126 The Vid30 Complex Regulates Glucose Repression and Derepression in Saccharomyces cerevisiae.

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The unicellular fungus Saccharomyces cerevisiae has evolved to utilize a wide range of carbon sources. Growth upon its preferred carbon source, glucose, results in the inhibition of gluconeogenic processes through highly co-ordinated mechanisms involved in carbon sensing, protein degradation, and transcriptional inhibition. This phenomenon, known as glucose repression, is maintained by a complex, tightly-regulated signaling network that rapidly responds to changes in environmental and intracellular sugar availability. For example, in the presence of abundant glucose, the gluconeogenic enzyme, fructose-1,6-bisphosphatase (FBPase), is ubiquitinated and degraded via the Vid30 Complex (Vid30c), an E3 ubiquitin ligase, thereby preventing gluconeogenesis. Active glycolysis also promotes the nuclear localization of the transcriptional repressor, Mig1, which binds to the promoter regions of gluconeogenic genes to prevent their transcription. When glucose becomes limited, Mig1-mediated gene repression is alleviated via differential phosphorylation of Mig1 by Snf1 kinase, resulting in disassociation of the Mig1 repressor complex and the upregulation (derepression) of glucose-repressed genes. Here, we use qRT-PCR analysis to show that Vid30c subunits are required for both the efficient repression of glucose-repressed genes and their subsequent derepression in the presence of non-fermentable carbon, like ethanol. Additionally, Mig1 migration profiles visualized via SDS-PAGE Western blotting suggest that the carbon source-dependent phosphorylation of Mig1 is also Vid30c-dependent under both carbon source conditions. In combination, these results describe a new role for the Vid30c in the transcriptional regulation of the glucose repression mechanism.

127 Cellular mechanisms of longevity by dietary restriction revealed by large-scale competitive-aging screens

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Dietary restriction—a reduction in calorie intake without malnutrition, or substitution of the preferred carbon or nitrogen source—is arguably the most promising nonpharmacological intervention to delay the onset of age-related diseases. Yet, only few genetic regulators mediating the cellular response to dietary restriction are known, and the question remains which other regulatory factors are involved. Here, we will present the identification of 473 yeast gene-deletion strains showing diminished or enhanced extension of lifespan, based on the high-resolution screening of the chronological lifespan under two nitrogen-source regimens. Functional analysis of such dietary-restriction genetic factors reveals novel processes underlying longevity by the nitrogen source quality, which has also allowed us to generate a prioritized catalogue of transcription factors orchestrating the dietary restriction response. Importantly, deletions of transcription factors Msn2, Msn4, Snf6, Tec1, and Ste12 result in diminished lifespan extension and defects in cell cycle arrest upon nutrient starvation, suggesting that cell-cycle regulation is a major mechanism of chronological longevity. Strikingly, STE12 overexpression is enough to extend lifespan, linking the pheromone/invasive growth pathway with cell survivorship. We have also interrogated the natural variability of longevity by dietary restriction in different populations; we will present evidence of hierarchical modularity in the signaling pathways underlying the dietary-restriction response. Our global picture of the genetic players of longevity by dietary restriction in the budding yeast highlights intricate regulatory cross-talks in aging cells.

128 Identifying Genes Required for Saccharomyces cerevisiae Growth in Mucin.

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The human gut microbiome is a vast ecosystem of microorganisms that play an important role in human metabolism, immunological function, and even inflammatory gut diseases. Metagenomics research on the human gut microbiome has demonstrated the presence of DNA from dietary yeast species like Saccharomyces cerevisiae. However, it is unknown if the S. cerevisiae detected in metagenomics studies is solely from dead dietary sources or if they can live and colonize the human gut like the closely related Candida albicans. While S. cerevisiae can adapt to low oxygen and acidic environments, it is currently not known whether it can live off or metabolize mucin, the primary carbon source found in the mucus layer of the human gut. Mucins are large, gel-forming, highly glycosylated proteins that make up the majority of carbohydrate sources in the gut mucosa. We determined that S. cerevisiae can utilize and adapt to growing in mucin as the major energy source in liquid media. We also observed a significant reduction in cell size when comparing S. cerevisiae cells grown in mucin to cells grown in the absence of mucin or in glucose media. We showed that an aspartyl protease named Yps7, part of a family containing known homologues to mucin-degrading C. albicans proteins in S. cerevisiae, is important for growth on mucin media. Additionally, to identify pathways required to grow on mucin, we conducted a chemogenomics screen using strains from the Saccharomyces cerevisiae Deletion Mutant Array and identified 28 genes that are important for S. cerevisiae growth on mucin media. These genes ranged in biological process including mitochondrial function (LPD1, MRS2, MZM1), protein degradation (GRR1, VPS27), transcription and RNA processing (DEF1, RAI1), signalling (SNF1, SOK1), among others. Out of these 28 genes, there were only two genes that, upon their deletion in S. cerevisiae, demonstrated a growth defect specifically on mucin media: CCM1 (a mitochondrial rRNA binding protein) and the uncharacterized YCR095W-A. Importantly, this project serves as the initial step towards establishing if our most common dietary fungi can survive in the mucus environment of the human gut.

129 SNPs Analysis Of MALR Transcription Factor In Beer Brewing Yeasts

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For maltose fermentation, budding yeast Saccharomyces sp. requires and utilizes transporters (MALT), maltases (MALS) and transcription factors (MALR) collectively encoded by the MAL gene loci. Recently, we have reported that the MALR gene (MAL73) in sake brewing yeast, which does not have maltose fermentation ability, is functionally impaired by a single nucleotide polymorphism (SNP) (1). This finding prompted us to explore SNPs of other MALR genes in a wide range of industrial yeasts. To date, Gibson et al. (2) have reported the sequence of several functional MALR genes (MAL23, MAL43, and MAL63). On the other hand, other MALR genes (MAL13 and MAL33) seem to be functionally irrelevant to maltose fermentation under laboratory conditions (3, 4).

In this study, we focused on MALRs in beer brewing yeasts, having high maltose fermentation ability. MAL23-, MAL43- or MAL63-like MALR genes in ten beer brewing yeast strains were analyzed, and fifteen distinct types of MALR were found in total. Intriguingly, some of those were found to be non-functional while the tested beer brewing yeasts showed high maltose fermentation abilities. The comparative analysis between the functional and non-functional MALR genes revealed that Ser321 is crucially important for MALR activity. Ser321 in functional MALRs was found to be substituted by proline in non-functional MALRs. Ser321→Pro replacement by genetic manipulation in functional MALR led to loss of function. Conversely, Pro321→Ser replacement in non-functional MALR led to gain of function. Attempts are under way to elucidate the biological implication why beer brewing yeasts have these types of non-functional MALRs.

(1) Ohdate et al. (2018) PLoS One 13: e0198744.

(2) Gibson et al. (1997) Genetics 146: 1287-1298.

(3) Charron et al. (1986) Mol. Cell. Biol. 6: 3891-3899.

(4) Chow et al. (1989) Mol. Gen. Genet. 217; 60-69.

130 Phenomic Screen Implicates the Yeast Lysine Acetyltransferase NuA4 in Regulation of Glycogen Synthesis and Mitochondrial Morphology through the PKA Inhibitor Bcy1.

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Cellular metabolism is tightly regulated by a myriad of signaling pathways, including by the post translational modification lysine acetylation. While in many cases lysine acetyltransferase (KAT)-dependent acetylation of enzymes impacts their activity, there is also growing evidence that lysine acetylation can change protein localization. As the Saccharomyces cerevisiae KAT complex NuA4 has been implicated in a variety of metabolic processes here we explore whether NuA4 is doing so through regulating the localization and/or abundance of metabolic proteins.

We performed a high-throughput microscopy screen with over 400 metabolic proteins and identified 23 proteins whose localization and/or protein levels changed upon deletion of the NuA4 scaffolding subunit, EAF1, including 3 proteins required for glycogen biosynthesis and 14 proteins associated with the mitochondria. We determined that in eaf1Δ cells the transcription of glycogen biosynthesis genes is upregulated resulting in increased proteins and glycogen production. Further our study shows that in the absence of EAF1 or in a mutant of catalytic domain of NuA4, esa1-ts, mitochondria are hyperelongated, chaotically distributed but remain functional. Quantification of the mitochondrial structure indicated that mitochondrial volume is increased approximately 3-fold in eaf1Δ cells relative to wildtype cells. Further, the increase in volume is associated with an increase in mitochondrial function as measured by extracellular flux analysis and the cells with increased mitochondrial volume are less sensitive to a mitochondrial inhibitor. Despite aberrant structure and function, the mitochondria of eaf1Δ cells respond similarly to wildtype cells in response to environmental stress. Our work shows that mechanistically, both the increased glycogen synthesis and mitochondrial structure change in an eaf1Δ cells are dependent on Bcy1, the yeast negative regulator of PKA. Surprisingly we find that in the absence of EAF1, Bcy1 localization changes from being largely nuclear to cytoplasmic. We are presently pursuing if the change in localization or metabolic regulation is due to potential acetylation sites on Bcy1 or other mechanisms.

As NuA4 is highly conserved with the human Tip60 complex, our work may prove to be directly transferable to human disease biology, revealing new avenues to investigate the role of Tip60 in metabolic regulation and by extension the deregulation that may occurs in the disease state.

131 Reprogramming of Candida glabrata epithelial adhesin ligand binding specificity by exchange of variable structural motifs

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Adhesion of fungal pathogens to host tissue is crucial for colonization and offers a promising target for drug development. In the human pathogenic yeast Candida glabrata, the family of lectin-like epithelial adhesins (Epas) represents the most prominent group of the more than 60 adhesins present in this organism. We are studying the structure-function relationships of Epa adhesion domains (EpaA), in order to understand how conserved and variable structural motifs contribute to functional diversity. Our previous studies have revealed several structural hot spots within the EpaA binding pocket that could be involved in programming glycan binding affinity and specificity, including two calcium binding loops, CBL1 and CBL2, and three loop forming regions, L1 to L3. To further understand the precise contribution of each of these highly variable structural motifs to ligand binding affinity and specificity, we have performed a structure-based mutational analysis. For this purpose, we have constructed chimeric Epa variants with exchanged CBL2 motifs and/or L1 loops using various donor and receiver EpaA domains. Chimeric variants were characterized by large-scale glycan array analysis and binding studies to human epithelial cells. Our data show that both CBL2 and L1 motifs are central elements for programming ligand binding specificity and host cell binding. Moreover, many of the chimeric variants exhibit novel functionality that are not shared by the donor or receiver domains. In summary, our study shows that the function of epithelial adhesins can be reprogrammed by modifying variable structural motifs and provides a possible mechanism for the evolutionary diversification of epithelial adhesins.

132 Constructing and Utilizing Transcriptional Regulatory Networks for Understanding and Redesigning Pathways in S. cerevisiae

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In recent years there have been many advances towards a bio-based economy, an economy based on the production of chemicals and building blocks from sustainable resources using biological and biochemical processes. Cell factories created by engineering metabolic pathways can convert renewable feed-stocks such as wood, starch, biomass and waste into fuels, chemicals, food ingredients and pharmaceuticals. Many food, pharmaceuticals and cosmetic ingredients are extracted from plants where seasonal dependent growth can cause supply depletion and extraction methods can be expensive. There is therefore much interest in developing cellular biocatalysts to produce direct replacements for specific chemicals as well as new advanced bioproducts that have properties superior to existing products.

In response to environmental changes the cellular functions are reprogrammed. Transcription factors play an essential role in this regulation. Through physical contact with either proteins or DNA the transcription factor alters the gene expression. Reconstruction of transcriptional regulatory networks require in depth understanding of these protein-protein or protein-DNA interactions. Because the targets of a transcription factor may differ depending on environmental conditions, different growth conditions will enable us to find the responses of each transcription factor. Using CRISPRi and the Transcriptional Regulatory Networks we can alter the gene expression of whole pathways. This we do to build better cell factories that can utilize the renewable feedstocks that can be used to produce high value chemicals.

133 Regulatory interaction between TOR and lncRNA guides activity of amino acid transporters BAP2 and TAT1 in S. cerevisiae

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Precise TOR based mechanism underlying the role of long non coding RNAs (lncRNAs) in regulation of amino acid uptake is largely unknown. lncRNA controls the expression of nearby protein-coding gene/s. We combined molecular and functional studies in S. cerevisiae to illustrate TOR dependent regulation of a locus consisting of two key amino acid transporters (AATs) BAP2 and TAT1 and an antisense lncRNA XUT_2F-154. We observed that AATs and lncRNA shows anti correlated expression pattern upon TOR inhibition. Reducing the transcription of lncRNA enhances the activities of AATs and decreases sensitivity towards TOR inhibitors. Interestingly, the expression of lncRNA is derived by BAP2 biphasic promoter upon TOR inhibition. Basically, inhibition of TOR changes nucleosome occupancy at both ends of BAP2 promoter to favor the transcription of lncRNA. lncRNA changes local chromatin environment to decrease the transcription of BAP2 and TAT1. Further studies are being undertaken to illustrate epigenetic factors involved in regulation process. This is pioneer report showing TOR mediated regulation of lncRNA governed amino acid uptake and hence recommends for new regulators that can be explored in development of novel therapeutic intervention strategies for fungal pathogenesis.

134 Yeast Biosensors To Detect Environmental Pollutants Into Effluent Waters.

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Due to human activities, such as farming, industry, untreated waste waters, many chemical compounds are released into the environment contaminating water, air and soil. These compounds constitute a threat for nature but also for human health.

Monitoring pollutants into aquatic resources is currently a major concern for many governmental organizations, and several European directives ask for accurate figures.

Pollutants are traditionally monitored by expensive and time-consuming analytical techniques, such as gas or liquid chromatographies associated with mass spectrometry analysis. Alternative methods based on biosensors are currently developed. The principle of these methods is based on the use of living cells sensing a pollutant at a cellular or molecular level coupled with the conversion of this molecular event into measurable analytical signals. Yeast biosensors appear to be a powerful tool into this field of investigation, as yeast is very robust, sensitive, easy to genetically engineer, and very cheap.

We want to develop new low cost biosensors by combining yeast to layered materials in order to improve yeast survival and sensitivity, and to detect and trap pollutants at the same time.

As a first step, we are developing a reporter system based on luminescent and/or fluorescent signals generated by proteins encoded by reporter genes described into the literature. These reporter genes will be turned on in presence of an identified pollutant. Then, we want to determine the best encapsulation medium to preserve yeast activity, sensitivity, and survival. Our ultimate goal is to associate this encapsulated biosensor yeast with layered materials, and then, to monitor their survival and their capability and sensitivity to detect pollutants in effluents.

135 Genetic Screening for New Factors Affecting NMD in S. cerevisiae.

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Nonsense-mediated decay (NMD) is a post-transcriptional process affecting the stability of mRNAs. Its role in regulating expression extends beyond the canonical recognition of premature stop codons. We are particularly interested in how NMD may modulate other response pathways following cellular stress. The factors affecting the NMD pathway in Saccharomyces cerevisiae have not previously been exhaustively identified by genetic or biochemical approaches. We aim to identify novel factors affecting the NMD pathway in yeast by utilizing the BY4741 yeast deletion collection and Scan-o-matic (Zackrisson et al., 2016), a novel high throughput microbial phenotyping platform. Two separate pathways that are known to be affected by NMD were screened sequentially. In the first screen, we exposed the deletion library to an elevated concentration of Cu2+ and identified mutants with increased tolerance. It is known that NMD targeting the CTR2 mRNA, encoding a copper transporter, affects fitness in high Cu2+ concentrations. In the second screen, a conditionally lethal synthetic construct depending on the NMD status of the cells was created based on the regulatory sequence of the CPA1 mRNA, another known NMD target, and is being introduced in the entire deletion collection followed by phenotyping. The mutants that show elevated tolerance to copper and increased sensitivity to toxin are likely to affect NMD and will be examined further using mRNA stability and transcription rate studies.

Reference:

Zackrisson, M., Hallin, J., Ottosson, L.-G., Dahl, P., Fernandez-Parada, E., Ländström, E., Fernandez-Ricaud, L., Kaferle, P., Skyman, A., Stenberg, S., et al. (2016). Scan-o-matic: High-Resolution Microbial Phenomics at a Massive Scale. G3 GenesGenomesGenetics 6, 3003–3014.

136 High-throughput strain construction using CRISPR-Cas9 and Selective Ploidy Ablation - Employing the yeast mutant libraries for metabolic engineering purposes

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Saccharomyces cerevisiae is an indispensable host for bioproduction of chemicals, fuels and pharmaceuticals. However, many yeast cell factories still suffer from low yields, which limits their industrial application. To increase production, a metabolic engineering strategy is often applied. This may not always be a straightforward task, as it requires either a priori knowledge of genes involved in the synthesis and control of the desired compound, or accurate predictions from genome scale modeling. Additionally, the yeast genome still contains a number of genes of unknown function, limiting the possibility to predict their influence on a metabolic pathway. In this regard, the yeast genome-wide mutant libraries are a great resource available to the yeast research community, as they can provide insight on the impact of specific genes on a metabolic process. Enabling efficient introduction of a biochemical pathway to these libraries would thus allow genes that are key for improved production to be identified. Here we present CRI-SPA (CRISPR/Cas9-Selective Ploidy Ablation) – an automated, mating-based method for high-throughput strain construction. We show the results of a proof-of-principle study in which CRI-SPA is used to introduce a genetic deletion to the yeast deletion collection with high efficiency and in just a few days' time. Furthermore, using the same library of mutant strains, we also apply the method to identify strains with improved production of compounds downstream of the Shikimate pathway, from which many industrially relevant chemicals are derived.

137 Structural and functional analysis of Wsc-type cell wall sensor proteins in Saccharomyces cerevisiae

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In Saccharomyces cerevisiae, the maintenance of cell wall integrity is governed by a family of sensors that include the Wsc- and Mid-type plasma-membrane spanning proteins. These sensors are supposed to detect mechanical forces occurring on the cell surface due to environmental stress or during vegetative growth and to target the highly conserved Cell Wall Integrity (CWI) signaling pathway to elicit appropriate responses. The Wsc-type sensors are characterized by an extracellular N-terminal cysteine-rich domain (CRD), which is essential for function, but structural information of this domain has been lacking so far. Here, we provide a high-resolution crystal structure of the Wsc1 CRD, which shows the presence of four conserved disulfide bonds that stabilize the compact domain folding and underpin their previously reported importance for sensor function and localization. Our structure further reveals an unusual high number of aromatic surface residues that can be grouped into three solvent-exposed clusters. These aromatic surface clusters are conserved in other S. cerevisiae CRDs, suggesting a functional role e.g. an involvement in the previously observed sensor-clustering due to hydrophobic interactions. In our current experiments, we are testing this hypothesis by mutational analysis.

138 Reversible Microhomology-Mediated Short Segment Duplication (MHSSD) Generates High Genetic Divergence

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Genome instability including changes in nucleic acid sequence, chromosomal rearrangements or aneuploidy, occurs rarely in general owning to various mechanisms safeguarding genome stability1. In contrast, tandem repeats pre-existing in the genome are recognized as mutation hotspots, with their repeat copy number expanding or contracting frequently2. A long standing question is that how tandem repeats are formed initially - i.e., how the birth of tandem repeats is achieved.

Here, by isolating genetically unstable mutants resistant to rapamycin in fission yeast, we identified unique mutation alleles (reversible, short DNA segment duplications) in ssp1, a gene encoding the calmodulin-dependent kinase, that are responsible for the unstable drug resistance. These mutations represent de novo formation of tandem repeats starting from a unique DNA segment flanked by microhomology (several base pairs in length) – here named as microhomology-mediated short segment duplication (MHSSD). We further show that this intrinsic genetic instability in ssp1 is modulated by DNA replication/damage repair processes. In a separate genetic study, we found MHSSD mutations in transcription regulator genes yox1 and lsk1, responsible for suppression of a growth defect caused by the cnp1-H100M mutation, demonstrating that MHSSD was not limited to the ssp1 locus or the rapamycin treatment condition.

Because of high abundance of microhomology-pairs throughout the genome (~80 pairs/kb), we postulate that MHSSD may occur prevalently. Consistently, by comparing whole genome sequencing datasets of 57 natural pombe isolates with the reference genome3, we identified 138 new sites that fit the criteria of MHSSD occurrence or reversion. These sites are distributed broadly throughout the genome, supporting the prevalence of MHSSD. Furthermore, by PCR amplification of some of these sites in a single colony and super-deep sequencing (>1x106 coverage) of the PCR products, we show that MHSSD occurs de novo within the relatively short duration of colony formation from a single cell.

Together, these results support the model that a new form of genome instability –MHSSD occurs frequently and reversibly at numerous sites throughout the genome, causing high genetic variations that can be functionally beneficial such as adapting to environment fluctuations and may facilitate evolutionary divergence.

139 Coordination Of Protein Homeostasis With Cell-size In Budding Yeast.

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Protein homeostasis is tightly coordinated with cell growth and size. In larger cells, transcriptional and translational machineries are more abundant and the production of proteins increases accordingly. While well suited for proteins that need to be maintained at a constant concentration, this mechanism imposes a problem for DNA-binding proteins, which on the contrary are required at a constant DNA-to-protein stoichiometry. Indeed, using live-cell fluorescence microscopy in the model organism S. cerevisiae, we find that the amount of histones increases only weakly with cell-size. As a result, the concentration relative to the total proteome decreases with increasing cell-size. This raises the question of how cells achieve to produce histones in proportion to DNA content rather than cell-size.

To identify the cell-size dependence of histone transcript concentrations, we controlled cell-size through expression of the cell-size-regulator Whi5 with a hormone-inducible promoter, resulting in steady state populations with a four-fold range of mean cell-sizes. Using RT-qPCR, we find that for control genes such as ACT1, the mRNA concentration relative to total or ribosomal RNA stays constant with increasing cell-size. In contrast, the relative concentration of histone mRNA decreases in inverse proportion to cell volume.

Next, we investigated by metabolic labeling of RNA whether changes in synthesis rate or degradation rate play a role in the cell-size-dependence of histone homeostasis. Initial results show a decrease in the relative synthesis rate of histone mRNA compared to control genes with increasing cell size, suggesting that the distinct cell-size-dependence of histone concentration is achieved at the level of transcription rate. This hypothesis is further supported by our finding that histone promoters driving the expression of a fluorescent reporter are sufficient to achieve a decrease of reporter mRNA concentration with increasing cell size.

140 Nutrient Signaling During Cell Cycle Progression.

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In Saccharomyces cerevisiae, nutrients act both as fuel and as signals for growth and the cell division cycle. The major checkpoint of the cell cycle in late G1 phase, called START, is defined as the cell cycle commitment point up to which cells respond to mating factors. According to the current model, not only mating factors, but various other signals, including nutrient signals, are also integrated up to the START point. However, evidence is accumulating that this model is incomplete and there is also crosstalk between nutrient signaling and the post-START cell division cycle.

Here, we aim to investigate the crosstalk between metabolism and the cell cycle with focus on S-phase and G2/M regulation. To this end, we employ fluorescent microscopy to enable single cell analysis of cells growing in a microfluidic cultivation platform, where we can control nutrient supply. In this set-up we can analyze the cells' response to nutrient depletion in consideration of their cell cycle phase. For example, we examine S-phase duration after nutrient depletion by monitoring production of the fluorescently tagged histone Htb2, and the G2/M transition by quantifying the kinase Swe1.

We found clear evidence that post-START cells can exhibit different cell-cycle related responses to nutrient deprivation. Both S-phase and the G2/M transition can be prolonged and possibly arrested during starvation. Further research will elucidate how nutrient signaling regulates specific aspects of progression through the cell cycle.

141 D-Xylose-assimilating Novel Yeast Species Isolated from the Gut of the Termite Macrotermes bellicosus

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Symbiotic relationships between insects and their gut-residing yeasts can have great impact to the ecosystem they reside in, reaching to human activities such as agriculture. For example, the relationship between the wasps Vespa crabro and Polistes spp. with the yeast Saccharomyces cerevisiae is crucial to the fermenting potential of grape harvests. Our aim is to explore whether such insect-yeast symbioses have evolved in other lineages and identify their nature. Specifically, we focused on the fungus-growing termite Macrotermes bellicosus (Termitidae: Macrotermitinae), an attractive model to study such interactions. We hypothesized that gut-residing yeast species can assist the termite in the degradation of complex plant fibers and lignin, a trait with great potential in the biotechnology industry. We collected individual M. bellicosus minor workers termites from four different colonies in the Comoé National Park, Côte d´Ivoire. Because of their origin and the underrepresentation of yeast isolates from Africa, we considered the termites as a valuable source for potential new yeast species. Termite guts were dissected out and smeared on yeast-selective medium, we identified yeast isolates by sequencing the ribosomal DNA (rDNA) ITS1 and D1/D2 regions and subsequent BLASTn alignment. We found that the isolates belong to Meyerozyma caribbica and Pichia caribbica, two novel subspecies of Candida quercitrusa and one new species within the Barnettozyma spp. clade. Lastly, we characterized the three novel isolates regarding growth on different carbon sources and their morphology by colony formation and microscopy. Their utilization of plant-derived pentose sugars, such as D-xylose, points towards a potential symbiotic role within the termite gut as they provide enzymes to degrade plant fibers. We are currently sequencing the yeast genomes to gain further insights into their metabolic routes.

142 Either Rap1 or Cdc13 Can Protect Telomeric Single-stranded 3’ Overhangs from Degradation in vitro.

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Telomeres are specialized DNA-protein structures that cap the end of linear chromosomes, preventing chromosome fusions and degradation of the DNA from the ends inward. The telomeric DNA tract is constituted by repeated G-rich sequence that commonly ends in a single-stranded (ss) 3’ overhang. The 3’ overhang plays a very important role in the maintenance and overall protection of the telomere. Changes in the length of the 3’ overhang are associated with loss of protection and telomere attrition, leading to replicative senescence and the formation of cancerous cells. Little is known about the protection of the 3’ overhang against the several exonucleases present in the cell. In this work, we developed an in vitro 3’ DNA end protection assay to study how Naumovozyma castellii Cdc13 and Rap1 proteins protect against 3’ exonucleolytic degradation by Exonuclease T. Given the homogenous telomeric repeat DNA sequence of N. castellii we were able to study the protection of the proteins at their exact binding site relative to the 5’ end. Our results show that the ssDNA binding protein Cdc13 could protect the 3’ overhang when bound directly adjacent to the 5’ end, extending a protection at least 5 nt beyond its minimal binding site (MBS). When bound adjacent to the 5’ end, the double-stranded (ds) DNA binding protein Rap1 could exert a protection 1-2 nt into the ssDNA. Rap1 has shown to bind across the ds-ss junction when its MBS contains 2 nt of ssDNA, strikingly this binding provides a strong protection of 6 nt into the 3’ overhang. These results show the mechanistics of the protection provided by the telomere binding proteins to maintain the integrity of the 3’ overhang and highlight the possibility of Rap1 to maintain a 3’ overhang when the Cdc13 protein cannot bind the telomere.

143 Global Sequencing of Circular DNA Reveals Loss of Genetic Variation and the Role of Replication Origins for Maintenance of Circular DNA in Aging Yeast Cells

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Circular DNA of chromosomal origin is a much overlooked group of genomic structural variants. To investigate the fate of circular DNA in aging cells, we purified circular DNA from 8 populations of young cells and their aged descendants and sequenced their circular DNA. We obtained a robust mapping of circular DNA by aligning reads probabilistically to the yeast genome and quantified circular DNA through internal plasmid controls. Our results reveal that young cells in average have 62 different circular DNA per 10e6 cells. Most circular DNA are lost as cells divide and only 6.3% are retained in cells as they age. Circles present in both young and old are characterized by replication origins, suggesting that this element is essential for retention. Among circles that persist as cells age are telomeric Y' circles, rDNA circles and circles formed from low copy repeat regions such as the hexose transporter genes HXT6 and HXT7. The number of different circular DNA is higher in young cells than old cells, but old cells have an overall higher copy number level of circular DNA caused by accumulation of rDNA circles (log2 Fold-Change = 6.88 ± 4.02). In conclusion, our results show that cells lose circular DNA genetic heterogeneity and accumulate telomeric and rDNA circles as they age.

144 Gln3 Links Nitrogen Assimilation With Fluconazole Resistance In Candida glabrata.

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Fungi are a major group of eukaryotic organisms with an estimated richness ranging from 3.5 to 5.1 millions of species. From these, only a small fraction has the capacity to cause invasive fungal infecions, as is the case for Candida glabrata. Infections with C. glabrata are usually treated with antifungal agents like fluconazole. However, there has been an increase in the number fungal infections with varying levels of intrinsic or acquired fluconazole resistance. Nitrogen assimilation has been well studied in various fungi, like in the yeast S. cerevisiae or in the filamentous fungi Aspergillus nidulans and Neurospora crassa. Recently, our group described that Gln3 has a fundamental role in nitrogen assimilation in C. glabrata. This GATA factor regulates some genes related to the transport and assimilation of ammonium and proline i. e. the ammonium permease, the general amino acid permease and the glutamine synthetase; encoded by MEP1, GAP1 and GLN1 genes, respectively. Nevertheless, the complete role of Gln3 in C. glabrata nitrogen regulation is not well understood. This prompted us to investigate the global contribution of this key transcriptional regulator of nitrogen assimilation using high throughput technology (RNA-seq). We compared the transcriptomes of a parental (WT) and a gln3 mutant strain grown in a standard minimal media with ammonium as sole nitrogen source. A total of 4,415 genes were detected and from these, approximately 50% were found as Differentially Expressed Genes (up-, or down-DEG) in the WT vs the gln3 mutant strain. The accuracy of the transcriptomic data was validated through the quantification, by qPCR, of 15 randomly selected genes. A detailed analysis of our RNA-seq data in combination with in-vivo experiments uncovers an unknown link between the transcriptional activator Gln3 and the main drug efflux transporters, Cdr1, and Cdr2 required for cell detoxification. This work opens a new area for future research regarding the relationship between nitrogen assimilation and fluconazole resistance.

145 NADPH deficiency reveals a tradeoff between adaptation and persistence under oxidative stress

Basile Jacquel and Gilles Charvin

IGBMC

Under oxidative stress, cells evolved by rerouting carbon in the pentose-phosphate pathway (PPP) to supply NADPH synthesis, required to fuel both antioxidant machineries and reductive biosynthesis. However, the physiological role of this metabolic rerouting in oxidative stress response is largely unknown. Here, combining single-cell time-lapse microscopy, mass spectrometry and mathematical modeling, we showed that PPP inhibition limited NADPH production. This limitation abolished both redox adaptation and growth of budding yeast cells under H2O2 in a PKA-dependent manner. However, this NADPH deficiency also led to an increased tolerance to oxidative stress, suggesting that those cells entered in a state of persistence. This high tolerance was dependent on an upregulation of the Yap1-regulon. Moreover, forcing growth in those cells under stress led to their death. Together, our results reveal a tradeoff between growth and redox homeostasis that is controlled in a PKA-dependent manner.

146 Replicative Senescence In S. cerevisiae: A New Tool For A New Approach

Théo Aspert and Gilles Charvin

IGBMC

Budding yeast is an interesting tool to study replicative senescence thanks to its asymmetric properties of division and the conserved mechanisms with higher eukaryotes. Indeed, 'Mother cells' experience a certain number of divisions before dying while daughter cells recover a full replicative potential. It is thought that ageing factors accumulate in the mother and are not transmitted to the daughter but the nature of these ageing factors, their toxicity and the mechanisms of segregation remain unclear.

In this context, our group developed a quantitative microscopy pipeline to track single mother cells throughout their entire lifespan.

We showed that 9 cells out of 10 display a sharp entry into senescence after a certain number of divisions, defined as an increase of the cell cycle duration. However, the cell-cell variability regarding the number of divisions before it occurs is high (Ferhmann et al. 2013).

Moreover, this dramatic increase of the cell cycle duration is correlated with an increase of the nucleus volume and preceded by an upregulation of nucleolar activity and nucleolar volume (Morlot et al. 2018) and an Extrachromosomal rDNA Circles (ERC) accumulation.

In other words, we claim that most of the S. cerevisiae single cells follow a common path of replicative senescence made of serial events, rather than multifactorial causes arising in a parallel manner. However, deciphering all the underlying cascade of events requires complementary bulk assays, which can lead to blurry dynamics and correlative results due to cell-cell variability relative to the senescence entry timing, with the usual "young VS old" approach.

Therefore, we developed a new methodology using microfluidics and FACS to culture mother cells to a certain age and sort them according to their senescent stage using specific markers, before performing biochemical assays on the different synchronized populations.

147 The Effects of Tbf1 and Reb1 on Telomere Function

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Subtelomeric regions contain protein binding sites that may vary between chromosomal ends. Since proteins bound to the subtelomeres as well as those associated to the telomeric repeats can govern the behaviour of telomeres, they account for some of the variability in the properties between all telomeres. Tbf1 and Reb1 are two essential yeast proteins that function as transcription factors on promoters of a multitude of genes. On gene promoters, both have roles in nucleosome exclusion, and Reb1 plays an important role in transcription termination for RNA polymerase I. They are also the most prevalent and ubiquitous proteins found on subtelomeres, although little is known about their function in these regions. The aim of this project is to better understand the contributions and effects of Tbf1 and Reb1 to telomere behaviour.

Previous results suggested that some properties of Tbf1 and Reb1 include effects on telomere length maintenance and anti-silencing (Arnéric and Ligner, EMBO reports, 2007; Berthiau et al., EMBO J, 2006, Fourel et al. EMBO J. 1999). These data were obtained using artificially constructed chromosome ends. In our study, we assess the contributions of Tbf1 and Reb1 by mutating their binding sites in otherwise wild type areas. This will allow us to evaluate changes in telomere length maintenance and the propagation of silencing that nucleates in telomeric repeats. Furthermore, the relevance of Tbf1 and Reb1 as transcription factors at the subtelomeres will be studied by measuring changes in TERRA expression in their absence.

By determining the relevance of Tbf1 and Reb1 to telomere function, this study aims to uncover the roles of subtelomeres at chromosomal ends, providing another tool to understand mechanisms essential for genome stability. I will be presenting our working model as well as preliminary results obtained.

148 Insights into the molecular underpinnings of Parkinson's disease: lessons from yeast

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Alpha-synuclein (aSyn) is a key player in Parkinson's disease (PD) and in a group of neurodegenerative diseases commonly known as synucleinophathies. In a living cell, proteins undergo a series of chemical modifications that regulate their structure, function, and localization. These are known as posttranslational modifications (PTMs). Recent findings indicate phosphorylation in several aSyn residues can modulate its aggregation and subcellular localization. Recent studies investigated the role of various families of protein kinases, such as the polo-like kinases, G protein-coupled receptor kinases, or casein kinases. In contrast, our understanding of the phosphatases involved in the dephosphorylation of aSyn is rather limited.

We found that aSyn can be glycated and acetylated, and that these modifications modify the aggregation and toxicity of aSyn in yeast. Importantly, our findings were also validated in mammalian cells, in drosophila and in mouse models of synucleinopathy, confirming their biological relevance.

In summary, using the powerful toolbox of Saccharomyces cerevisiae we are not only unravelling the molecular underpinnings of neurodegenerative diseases, but also opening novel avenues for therapeutic intervention in diseases for which we currently have no effective therapies.

149 Acute Glucose Starvation Induces Global Cytoplasmic Reorganization and Metabolic Reprogramming.

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The ability to tolerate and thrive in diverse environments is paramount to all living organisms. Specifically, many unicellular organisms, including yeast, spend a large part of their lifetime in starvation. Upon acute glucose starvation, yeast cells undergo drastic physiological and metabolic changes allowing them to establish a constant - though lower - energy level within minutes. Furthermore, both budding and fission yeast respond to acute glucose starvation by modulating the biophysical properties of their cytoplasm (Joyner et al., 2016, Munder et al., 2016). This response manifests in increased cytosolic rigidity and macromolecular crowding as well as enhanced liquid-liquid phase separation of proteins and RNA, while cytosolic diffusion is restricted. These changes to the bulk physical properties of the cytoplasm are expected to globally affect cellular function by broadly modulating protein-protein interactions. Yet, how and why the cell modifies these properties remains unknown.

To better understand the response of yeast cells to acute glucose starvation, we have characterized the cells' immediate metabolic response as well as the rapid changes to the biophysical properties of the cytosol. We observe that central carbon metabolites drastically deplete within seconds and find that a switch from fermentative to respiratory metabolism is needed to establish a new, though lower, energy level in S. cerevisiae cells as well as to enable survival of starvation. We also performed a screen to identify mutants that are unable to retain their cytoplasmic rigidity upon glucose starvation, and correlate our metabolic findings with the cells' ability to increase its cytosolic rigidity and decrease cytoplasmic diffusion.

References: Joyner et al., 2016. eLife 2016;5:e09376; Munder et al., 2016. eLife 2016;5:e09347

150 Uncovering the Crucial Property of 4-Deoxy-L-erythro-5-hexoseulose Uronate for the Utilization of Alginate, a Promising Marine Biomass

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Alginate that is a linear polyuronate in brown macroalgae and thus is a promising marine biomass. 4-Deoxy-L-erythro-5-hexoseulose uronate (DEH) is a monouronate formed through a digestion of alginate by exo-type alginate lyase. A bioengineered yeast Saccharomyces cerevisiae (DEH++) strain with the ability to utilize DEH has been developed for biorefinery systems 1). Thus, DEH is not only an important physiological metabolite but also a promising carbon source for the utilization of alginate in biorefineries1). Here, we present the crucial chemical property of DEH. In particular, we showed that DEH non-enzymatically reacts with specific amino groups at 30°C and forms other compounds, one of which was identified. In contrast, some amino acids were inert to DEH. Some of these inert ones were suitable nitrogen sources for the DEH++ yeast strain, while the reactive ones were poor nitrogen sources. These results clarify the reactive property of DEH, providing a basis for choosing a nitrogen source for the utilization of DEH and alginate in biorefineries as well as insights into the physiological utilization of DEH.

1. Matsuoka, F. et al. Crucial role of 4-deoxy-L-erythro-5-hexoseulose uronate reductase for alginate utilization revealed by adaptive evolution in engineered Saccharomyces cerevisiae. Sci. Rep. 7, 4206, doi:10.1038/s41598-017-04481-310.1038/s41598-017-04481-3 [pii] (2017).

151 Tracking Telomerase RNA via Inducible Tagging

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Telomerase reverse transcriptase extends the ends of linear eukaryotic chromosomes, thus counteracting their constant erosion and loss of vital genetic information. Tight regulation of telomerase synthesis and maturation is absolutely required for its proper function. Hence, dissecting telomerase life cycle and the layers of its regulation is essential for the development of new strategies against telomerase-positive cancer and diseases associated with telomerase defects, such as diskeratosis congenita and aplastic anemia.

Budding yeast telomerase consists of the catalytic subunit Est2, other accessory proteins and TLC1 RNA. Previous studies suggest that the newly transcribed TLC1 molecules undergo a complicated life cycle, which involves RNA maturation in the nucleolus and possibly a temporary export of TLC1 to the cytoplasm for the assembly of the telomerase holoenzyme. Indeed, TLC1 molecules can be detected by FISH both in the nucleus and in the cytoplasm. However, direct evidence of the cytoplasmic stage of telomerase assembly is lacking.

Due to a long half-life of telomerase RNA, it is technically challenging to distinguish between the new and old TLC1 molecules. To overcome this problem, we have applied an inducible tagging system which allows tracking newly synthesized telomerase RNA in living and fixed cells by fluorescent microscopy. This system involves recombination-mediated addition of the MS2 tag to TLC1, which can be induced by supplementing growth media with a drug. Using the MS2-GFP fusion protein in live-cell fluorescent microscopy experiments or the MS2-specific fluorescent probe in fixed cells, we can detect and track exclusively newly synthesized TLC1-MS2 RNA molecules. We have observed preferential cytoplasmic localization of new TLC1 molecules, indicating that early steps of TLC1 life cycle occur outside the nucleus. We are currently investigating the biological significance of such subcellular TLC1 trafficking and the factors involved in this process.

A similar strategy is also used for inducible TLC1 untagging, which makes only the old TLC1 molecules detectable. By tracking this subset of telomerase RNA, we can better characterize the process of TLC1 degradation.

Here, we are presenting our early results in applying an inducible tagging system for studying the TLC1 RNA life cycle.

152 Identification of FUS and OPTN Disaggregating Compounds and their Plasma Membrane Import Routes Using Yeast Based High-content Screening.

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The drug development for diseases that affect the Central Nervous System (CNS) should consider the transport of the compound through the human Blood Brain Barrier (BBB). Due to the protective function of this barrier, the entrance of xenobiotic molecules is restricted and occurs preferentially via membrane carriers present in the BBB cells. Hence, the development of methodologies for the study of how these compounds cross the membrane is essential for the development of drugs that efficiently target the CNS. Saccharomyces cerevisiae is a well-studied organism for which complete collections of gene deletions are available for high-throughput experiments, such various drug screening approaches. A strategy already well established in our group is a screening method for drug uptake using a library of single deletions of the genes encoding each of the non-essential S. cerevisiae plasma membrane transporters. Our rationale is to identify transporters responsible for drug uptake in the yeast plasma membrane and translate these findings to the orthologue transporters of the human BBB. In this context, we focus on the identification of novel compounds targeting a disease that affect the CNS: Amyotrophic lateral sclerosis (ALS). We have selected, based on hierarchical clustering method, 400 molecules that represent the diversity of the DIVERSet-EXP library (ChemBridge Corporation) and screened these for inhibitors of human OPTN and FUS aggregation in yeast-based high-content screens. We have identified promising compounds, which inhibit the aggregation of specific human proteins, and are now testing similar compounds for improved disaggregation activity. Once our best hits are confirmed, we aim to identify which carriers are responsible for their uptake into yeast cells. This knowledge will be used to identify putative human BBB transporters that could potentially promote the uptake of novel disaggregating compounds.

153 The Role of Crossfeeding in the Emergence of Purine Auxotrophs

Janis Liepins, Stivens Zolins, Zane Ozolina and Agnese Kokina

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Mutations are drivers of evolution, however, the exact mechanism how and under what circumstances they get fixed in particular organisms, are not well understood. Indeed, random mutations occur in every organism. In most cases these have no effect on phenotype. However, some mutations lead to phenotypical changes, for example, complete dysfunction of particular protein and/or pathway. Purine auxotrophy is such example – mutation that leads to dysfunction of most steps of purine de novo synthesis pathway, leads to purine auxotrophy. We know, that during evolution purine auxotrophy, have emerged numerous times in different organism groups. Parasitic plathelminths and intracellular parasitic protozoans are notorious examples of “naturally occurring” purine auxotrophs.

When mutation leading to auxotrophy of the microorganism occurs, there would be some time of co-existance of “wild type” and newly emerged mutants. If mutants are incapable of purine synthesis, then, supply of purine metabolites should be sustained by intake from the media – from host or from surrounding, prototrophic peers.

We tested if purine de novo synthesis mutants of budding yeast Saccharomyces cerevisiae can be sustained in prototrophic population by cross feeding. We determined the minimum necessary supply of purine metabolites for knockouts of adenine synthesis pathway. We estimated the potential of purine “excretion” from prototrophic strains. We set up prototroph – auxotroph crosfeeding experiments with test strains and measured the persistence of auxotrophic strain over time.

Our results demonstrate, that purine metabolite crosfeeding from the prototrophic wild type strain to purine auxotroph is limited. Our results imply, that emergence and sustain of eukaryotic purine auxotrophs most probably occurs due to supply of necessary purines from surrounding media (or host) rather than crosfeeding from their genetic peers.

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154 Role of Mutations in Purine de novo Synthesis Pathway in Cell Cycle Arrest and Stress Resistance Phenotype During Purine Starvation

Agnese Kokina, Kārlis Pleiko, Zane Ozoliņa and Jānis Liepiņš

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The loss of function of some biochemical pathway have direct effects related with stop of synthesis of particular metabolite. However, other pleiotrophic effects might arise during specific environmental context. To characterise potential effect particular mutation might have, several environmental contexts should be tested. Besides, starvation is typical state of microorganisms in the wild. Testing mutation effects on starving cultures can simulate their effects close to real life situations. Here we report on phenotypic effects observed in budding yeast purine auxotrophs during purine starvation. Purine auxotrophy is common feature of many budding yeast Saccharomyces cerevisiae laboratory strains. It is widely used as a marker for gene engineering (ade2 or ade8). Currently we know that S. cerevisiae ade2 and ade8 strains behave similarly when starved for purines. Their purine pool decreases, stress tolerance and cell size increases (Kokina et al. 2014). Yet, it is unknown how these mutants “choose” to initiate stress resistance phenotype during purine starvation. Moreover, question do all gene knockouts of purine de novo synthesis pathway equally contribute to stress resistance phenotype during purine starvation remain open.

We think that in addition to the well documented regulation within purine de novo synthesis pathway, it’s steps differently contribute to the organism’s fitness during starvation. We presume that not all mutations in different steps of purine de novo pathway equally contributes to creation of stress resistance phenotype and probably not all of them equally promote cell cycle arrest. We would like to understand if cells have general “plan” how to cope with decreasing intracellular purine pool or “the plan” is different for each knockout in the purine de novo synthesis pathway.

Thus far our data on purine starvation induced phenotype point to the turn on of rather general “survival program”. However, halt of cell cycle does not always correlate with increased stress tolerance and response to purine starvation is also strain dependent.

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155 Cell Cycle Regulation at the Level of mRNA.

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In order to live, cells need to adjust the balance of somatic versus replicative growth to environmental factors. While the checkpoint at the G1/S-transition (Start) is well described, it is less clear which properties are measured at other checkpoints. Baker's Yeast cells that are shifted to a poorer carbon source during S-phase of the cell cycle need to delay mitotic events, or risk producing genetically incomplete, or too small daughter cells. A plausible mechanism to ensure viable offspring is to measure biosynthetic capacity of the emerging daughter cell, i.e. whether it can produce enough protein to live independently. mRNA of the dominant mitotic cyclin Clb2 has been reported to localize to the bud, so that Clb2p concentration reflects the bud's biosynthetic capacity. Previous experiments relied either on fixed cells, i.e. without the possibility to observe a subsequent mitosis, or required light-dosages that would lead to cell cycle arrest. Using lattice light sheet microscopy and the MS2 system, we quantified and localized mRNA throughout the cell cycle in individual cells to investigate whether bud-localized CLB2 is required for mitotic entry. Our data suggests that CLB2 bud-localization occurs in most cells prior to mitotic entry, while additional mechanisms are required to warrant the robustness of this checkpoint. We thus correlated mRNA and protein abundance of Clb2, assessing how predictive mRNA abundance is for the activity of the promitotic Clb2/Cdc28-complex. In summary, our findings suggest that mRNA behavior is an important factor in the regulation of mitotic entry.

156 A Novel Phosphoketolase Assisted Circular Pathway for High Yield Precursor Supply in Yeast.

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A potential target for metabolic engineering approaches, to increase the TRY-metrices (titer, rate, yield) for a process, is a precursor metabolite. A very important precursor metabolite is acetyl-CoA, which can be converted into numerous different industrial relevant products. By increasing the flux towards the precursor metabolite, the flux towards its product can often be increased.

There exist several metabolic engineering strategies to increase the acetyl-CoA pool, however, they all suffer from the inherent limitation of carbon loss as CO2. This limitation results in wasted carbon and thus reduced product yields. This has a major impact for industries as one of the major costs for industrial fermentation is the cost of the substrate.

An alternative route for carbohydrate metabolism that does not result in carbon loss is the “bifid” shunt found in Bifidobacteria. This pathway utilizes the enzyme phosphoketolase that can cleave either fructose-6-phosphate (F6P) or xylulose-5-phosphate (X5P) to acetyl-phosphate (AcP). Heterologous expression of a phosphoketolase, together with a phosphotransacetylase, allows conversion of F6P or X5P to acetyl-CoA in yeast without the loss of carbon as CO2.

This route is still limited to producing only 1 acetyl-CoA per F6P or X5P without any carbon loss. By knocking out competing pathways and overexpression of a promiscuous phosphoketolase, which also can act upon sedoheptulose-7-phosphate (S7P), a cyclic pathway can be achieved that is generating 3 moles of acetyl-CoA per F6P without any carbon loss.

In our work, we have tested this pathway and shown that it is functional in both in vitro and in vivo, using Saccharomyces cerevisiae as a host.

158 A Novel Connection Between the Nuclear Pore Complex and Cell Cycle Regulation.

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CABIMER / CSIC - University of Seville - University Pablo de Olavide

Proper chromosome segregation during mitotic division is of pivotal importance in order to avoid the appearance of aneuploidies. There are many mechanisms, known as “mitotic checkpoints”, that ensure a proper DNA distribution during the cell cycle, such as the DNA damage checkpoint (DDC, which ensures genome integrity), the spindle assembly checkpoint (SAC, which controls correct attachment of all kinetochores to the spindle) or the spindle positioning checkpoint (SPOC, which avoids mitotic exit when the spindle has not been correctly positioned). Interestingly, in the budding yeast Saccharomyces cerevisiae, these checkpoints share the ability of arresting the cell cycle by activating the Bfa1-Bub2 complex and subsequently inhibiting the Mitotic Exit Network (known as MEN), a signaling cascade that leads to the completion of mitosis. Accordingly, the regulation of Bfa1-Bub2 activity is crucial to maintain genomic stability and cell cycle progression. How different intranuclear signals can modulate the inhibitory activity of the Bfa1-Bub2 complex, which is located in the cytoplasmic side of the spindle pole bodies (SPB), is still unclear. Here we demonstrate a novel interaction between Bfa1 and components of the nuclear pore complex (NPC). The characterization of this Bfa1-NPC interaction will shed new light into the mechanisms by which exit from mitosis is regulated.

159 A Graded Fission Yeast MAPK Response to H2O2 Requires Wis1 Thiol Inhibition and is Reversible by a Small Molecule.

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The human MAP Kinase Kinase (MAPKK) MKK6 is inactivated through the formation of an intramolecular disulfide, despite that MAPK signaling in the p38 pathway is activated by H2O2. Only one of the involved cysteines, located in the active site, is conserved in mammals, budding and fission yeast MAPKKs. Here we show that H2O2 nevertheless inactivates the fission yeast MAPKK Wis1 in a manner reversible by thiol reductant and dependent on the active site cysteine, C458. This cysteine is oxidized in vivo, and mutating C458 to serine boosted the Wis1 response to low levels of H2O2. The hypersensitivity of the wis1-C458S mutant to H2O2, but not osmotic stress, emphasizes the importance of a graded, dose-dependent stress-activated MAPK pathway response for H2O2 resistance. Further supporting inhibition of Wis1 activity by low levels of H2O2 targeting Cys458, we found that pretreatment with an allosteric human MAPKK inhibitor, INR119, binding near Cys458 in Wis1, enhanced the Wis1 response to low H2O2 by blocking Cys458-mediated inactivation both in vitro and in vivo. Together, our results demonstrate the importance of thiol inhibition in a graded MAPK stress response and the possibility of targeting this mechanism by a small molecule to potentiate MAPK signalling.

160 Metabolism is Tailored to Biosynthetic Needs During the Budding Yeast Cell Cycle.

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The cell division cycle is a complex, highly regulated process requiring coordination of multiple processes. Global, multi-level insight into the cell cycle, however, has remained minimal. Here we use the eukaryal model Saccharomyces cerevisiae to perform absolute quantitative multi-omics to map interactions of these different processes. We show that cell cycle effects on translation is a re-occurring feature, alongside transcriptional regulation of periodic proteins for controlling on demand synthesis. This impacts biosynthesis, with a third of enzymes showing dynamic abundance. Changes in metabolic activity is additionally supported by changes in the metabolome. Periodically phosphorylated proteins also show to be important for coordinating a wide range of activity on demand. Our top-down Systems Biology approach offers a framework for analysing complex processes, including how metabolism is reshaped to different biosynthetic needs. This could impact our understanding of metabolic changes associated with the transformation of non-growing cells to proliferating cancer cells.

161 Do Synonymous Mutations Have an Evolutionary Significance?.

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S. cerevisiae and D. discoideum are unicellular organisms diverged millions of years ago, yet they differ in many fundamental aspects, such as AT/GC content. Despite this divergence, they share many metabolic as well as developmental features. Thus, the fundamental question is how these organisms evolved similar physiological traits with vastly differing nucleotide composition? The commonality between nutrient-dependent differentiation processes between these two organisms is the formation of multi-cellular structures in response to Carbon and Nitrogen limitation. Interestingly, ammonia is a differentiation-inducing factor in both organisms. S. cerevisiae has three ammonium transporters, MEP1, MEP2, and MEP3, of which MEP2 acts as a transceptor and deletion of MEP2 alone is sufficient to abrogate the differentiation response. In contrast, D. discoideum has five ammonium transporters of which amtA is more similar to MEP2. Thus, we surmised that probing the functional similarity despite the evolutionary divergence would address how sequence space gets evolved to retain the functional similarity.

We observed that amtA does not complement the growth defect in S. cerevisiae caused by triple deletion (mep1mep2mep3Δ) at low ammonia (below 1mM ammonia) conditions. We isolated two mutant forms of amtA through random mutagenesis that complemented for the growth defect. Further analysis showed that the mutations in these two independent mutants are a combination of non-synonymous and synonymous substitutions. Mutant 1 has 3 non-synonymous and 2 synonymous substitutions, whereas mutant 2 has 2 non-synonymous and 1 synonymous substitutions. Mutant 1 requires all the 5 substitutions, and mutant 2 requires all the 3 substitutions for the complementation at low ammonia. Localization experiments showed that both wild type and mutant forms of amtA are translocated into the membrane, indicating that the bottleneck for functionality resides in the proper folding and assembly of the amtA in the membrane. This possibility is supported by the occurrence of synonymous mutations, which are known to play a role in proper folding. Observations that two independently isolated mutants have a total of three synonymous mutations which are integral to its function, further buttresses our claim. Thus, our observation strongly argues against the long-held notion that synonymous mutations do not have any evolutionary consequence.

162 Relationship of Storage Carbohydrates Accumulation With tRNA Synthesis in Saccharomyces cerevisiae.

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Intuitively we may think that differences in the tRNA levels are followed exclusively by changes in the translation rate. However, we observe that decreased RNAP III dependent tRNA transcription in RNAP III compromised cells [1] or the absence of Maf1, the negative regulator of RNAP III [2], elicit broad changes in fundamental metabolism in S. cerevisiae [3]. RNAP III activity indirectly affects processes such as transcription of HXT encoding glucose transporters [4], gluconeogenesis [1], glycolysis [3].

Our previous data confirmed a strong, negative correlation between RNAP III activity and enzymes abundance in the trehalose and glycogen synthesis pathways, which share common enzymes [3]. During logarithmic growth, metabolic overflow in maf1Δ leads to flux redistribution into the trehalose pathway and conversely, a diminished flux in rpc128-1007 results in disability to accumulate neither glycogen nor trehalose.

This study is the first attempt to elucidate the regulatory mechanism of trehalose synthesis de novo in yeast strains accordingly to RNAP III activity. Despite the diminished content of trehalose, we observed increased transcripts levels of several genes involved in storage carbohydrates metabolism in rpc128-1007 strain. To investigate the fate of mRNA in rpc128-1007 we performed immunofluorescence assay using common markers for stress granules and P bodies formation. Metabolic reprogramming, which occurs in rpc128-1007 during fermentative growth [3], is correlated with mislocalization of Dbp2, the RNA Helicase, which is involved in transcriptional control and glucose metabolism, and acts as an intracellular link integrating environmental nutrient conditions with regulation of glucose-repressed, Cys8-targeted genes across the genome [5, 6].

Evaluation of enzymes activities, Ugp1 (UDP-glucose pyrophosphorylase) and Nth1 (neutral trehalase), which belong to trehalose pathway, further followed by investigation on protein kinase A (PKA) activity - dependent levels of carbohydrates suggest, that the regulation of trehalose biosynthesis de novo, in the strains under study is regulated on the post-translational level. Acknowledgement: This research received funding from the National Science Centre Poland, grant no. 2012/05/E/NZ2/00583 for MA.

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163 From Phenotypes to Pathways: Global Analysis of Subcellular Compartment Morphology using Systematic Genetics and Single Cell Imaging

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We have developed experimental/computational pipelines which combine Synthetic Genetic Array (SGA) analysis, which automates yeast genetics, and high through-put microscopy for systematic and quantitative cell biological screens. One pipeline uses SGA to introduce fluorescent markers of key cellular compartments 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.

We have used this approach to explore endocytosis, a conserved process that mediates cellular uptake of nutrients and other molecules, including pathogens. For this project, we performed neural-network based image analysis of single cells to screen for genes that influence the morphology of four main endocytic compartments: coat proteins, actin patches, late endosome and vacuole. This unbiased approach identified 17 mutant phenotypes and ~1600 genes whose perturbation affected at least one of the four compartments. Many mutants were associated with multiple phenotypes, indicating that morphological pleiotropy is prevalent within the endocytic pathway. Morphological profiles composed of the 17 mutant phenotypes were correlated for functionally related genes, enabling the prediction of gene function. Incomplete penetrance was prevalent within the endocytic pathway, and single-cell analysis enabled exploration of the mechanisms underlying cellular heterogeneity, which include cell size, cell-cycle distribution, replicative age, organelle inheritance, and stress response.

164 Destabilization of EIF3a Renders the EIF3 Factor Vulnerable to Aggregation at Physiological Growth Conditions and Leads to an Early Formation of Stress Granules in Saccharomyces Cerevisiae Cells.

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Cells have elaborated a complex strategy to maintain protein homeostasis under physiological as well as stress conditions to ensure the smooth functioning of vital processes and producing healthy offspring. Impairment of one of the most important processes in living cells, translation, might have serious consequences including various brain disorders in humans. Here we report on a variant of the essential translation initiation factor eIF3a, Rpg1-3, mutated in its PCI domain that displays an attenuated translation efficiency and formation of reversible assemblies already at physiological growth conditions. Dynamic Rpg1-3 assemblies actively move not only within the mother and daughter cell area but are able to pass through the bud neck from the mother to the daughter cell by an active bud-directed movement (BDM). Energy, intact actin cytoskeleton and the molecular motor Myo2 appeared to be important for BDM as well as for the directionality of the foci movement within the mother and daughter cells. Interestingly, the ability to form assemblies is transmissible since all cells in the progeny of a single cell with Rpg1-3 foci do contain these foci although Rpg1-3 assemblies do not have an amyloid nature. Presence of other members of the eIF3 core complex in Rpg1-3 foci might be the reason why yeast cells do not recognize these assemblies as misfolded protein aggregates and let them be transferred into daughter cells. These results open the way to understand mechanisms yeast cells employ to cope with malfunction and aggregation of essential proteins and their complexes.

In our previous studies, we have established eIF3a as the marker of yeast stress granules induced by high-temperature stress at 46°C (hs-SGs) that harbor components of the translation machinery, mRNAs, and proteins affecting mRNA dynamics. At a moderate heat shock of 42°C, the wild-type eIF3a stays diffused in the cytoplasm since the translation is not arrested but slowed down only. When Rpg1-3 is the only source of eIF3a in cells, stress granules are formed already at moderate-temperature stress of 42°C due to the arrest of translation. 42°C-SGs are similar to hs-SGs in their composition, however, the dissolution of 42°C-SGs is much faster than that of hs-SGs. Our results demonstrate that affecting the function and structure of an essential translation component such as the eIF3 factor leads to sequestration of proteins and mRNAs even upon less severe stresses.

165 Loss of Control at G1/S Phase Border of Cell Cycle in swi6Δ Cells of S. cerevisiae Leads to Genome Instability through Replication Stress-induced DSBs and Rad51-mediated Illegitimate Recombination.

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The Swi6 protein from budding yeast is mostly known for its role in controlling expression of G1/S transition genes. However, Swi6 also protects cells against different stresses, including cell wall stress, unfolded protein stress, oxidative stress, and, as indicated by our recent genome-wide studies, against double-strand breaks in DNA. We showed that swi6Δ cells display symptoms of replication stress and inefficient DNA repair. In these cells DNA replication is slowed down due to limitations in abundance of factors involved in DNA synthesis. Thus DNA strands at the replication forks are exposed to possible damage for longer time. Moreover, the replication checkpoint is not fully activated in swi6Δ cells, which precludes proper DNA damage response. Replication block leads to DSBs formation, and subsequent DNA rearrangements, thus causing genome instability and increased cell mortality. Comparative genomic hybridization analysis revealed duplication of chromosome V in SWI6 deleted diploid yeast cells. Chromosome V contains PAB1 and SWI4, two previously reported multicopy suppressor genes of swi6Δ, which suppress its transcription-dependent phenotypes. Overexpression of these genes only partially reverses genome instability phenotype of swi6Δ. Since on chromosome V also RAD51 gene is located, we asked if overexpression of this gene supports swi6Δ cells survival during genotoxic stress. And indeed, it does. We also showed that in swi6Δ mutants Rad51 level is elevated while the level of Srs2 helicase/Rad51-translocase is diminished. Moreover, these cells accumulate complex difficult to resolve recombination intermediates. All these findings led us to conclusion that Rad51-dependent illegitimate recombination is the repair pathway responsible for both, cell survival and genome rearrangements in swi6Δ mutants.

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166 Efficient Vesicular Trafficking Protects Yeast Saccharomyces cerevisiae Genome from Fragmentation.

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With time, more and more cellular mechanisms are considered as significant for the genome maintenance. Results of our previous genome-wide studies had drawn our attention to vesicular trafficking as a process that contributes to genome preservation. Among 63 strains lacking various vesicular trafficking genes that were engaged in the protection of cells against stress of DNA double strand breaks or involved in spontaneous mutagenesis, we found several strains that showed highly increased DNA fragmentation revealed by the single cell gel electrophoresis technique (Comet assay). These strains exhibited also other phenotypes linked to genome instability. Firstly, they showed increased sensitivity to exogenous genotoxic stress (e.g. upon zeocin or MMS treatment). Secondly, in these strains, the recruitment of Rad52-YFP to DNA repair foci was decreased, which suggests impaired DNA double strand breaks recognition or defects in DNA repair. Thirdly, these strains displayed increased frequency of Rfa1-YFP spontaneous foci formation, as compared to control strain. Moreover, in contrast to WT control, the rate of Rfa1-YFP foci formation was not elevated significantly in response to genotoxic stress. Finally, the flow cytometry analysis of mutant cells stained with propidium iodide indicated DNA content aberrations. Collected data imply moonlighting role of vesicular trafficking proteins in cellular response to DNA damage.

This work was supported by NCN grant 2016/21/B/NZ3/03641

167 Deciphering the Crosslink Between pH and Calcium Homeostasis in Yeast

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Cells have developed a sophisticated sensing and signalling system to adapt and survive in response to a variety of environmental challenges, such as temperature, pH, osmotic pressure, and nutrient availability. As in other eukaryotes, yeast cells employ a complex calcium signalling network to regulate diverse cellular processes upon external stimuli. Several lines of evidence indicate that the glucose-induced activation of plasma membrane H+-ATPase is dependent on calcium signalling, which reveals an intimate connection between the pH and calcium homeostasis. However, the correlations between external pH, intracellular pH, and intracellular calcium metabolism is still unclear. In this work, we used Saccharomyces cerevisiae as a model system to establish the glucose-induced pH and TECC (Transient Elevation of Cytosolic Calcium) responses in a collection of mutant strains at different extracellular pH (pHe). The TECC response at pHe 7 in all the selected yeast strains were dramatically higher than that at pHe 5. Interestingly, the higher TECC response at pHe 7 also displayed the faster alkalization of cytosolic pH after glucose re-addition to glucose-starved yeast cells. In addition, the results also showed that the cytosolic calcium influx signal appeared before the alkalization of cytosolic pH, which was in line with the previous evidence which suggested that the plasma membrane H+-ATPase is calcium dependent.

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168 Cloning-free Multiplex CRISPR/Cas Genome Editing in Saccharomyces cerevisiae using T7 RNA polymerase-assisted guide RNA expression.

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Even for the genetically accessible yeast Saccharomyces cerevisiae, the CRISPR/Cas DNA editing technology has strongly accelerated and facilitated strain construction. Several methods have been validated for fast and highly efficient single editing events. While diverse approaches for multiplex genome editing have been described in literature by means of Cas9 or Cas12a endonucleases and their associated gRNAs (reviewed in Adiego-Pérez et al., 2019), multiplex genome editing remains less efficient and cumbersome. In these approaches, the gRNAs used to guide the Cas endonuclease to the editing site are typically expressed from plasmids using native PolII or PolIII RNA polymerases. These gRNA expression plasmids require laborious, time-consuming cloning steps, which hampers their implementation for academic and applied purposes. In this study, we explore the potential of expressing gRNA from linear DNA fragments using the T7 RNA polymerase (T7RNAP) for single and multiplex genome editing in S. cerevisiae. Using Cas12a, this work demonstrates that transforming short, linear DNA fragments encoding gRNAs in yeast strains expressing T7RNAP promotes efficient DNA editing. These DNA fragments can be custom-ordered, which makes this approach highly suitable for high-throughput strain construction. This work expands the CRISPR-toolbox for large-scale strain construction programs in S. cerevisiae and promises to be relevant for other, less genetically accessible yeast species.

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This project is funded by the Netherland Organization for Scientific Research (NWO), Building Block of Life funding.

170 Goldilocks and the Three Genotypes: Characterizing the Prevalence of Overdominance for Adaptive Mutations that Arise in Diploids

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When evolving diploids acquire mutations, those mutations are generally heterozygous. Some of these heterozygous mutations provide a fitness benefit, making the mutants more fit than their unmutated ancestral counterparts. When the heterozygous mutant (Aa) is more fit than either the homozygous mutant (aa) or homozygous ancestral (AA) genotype, that mutation is described as being overdominant. A well-known example of overdominance in human populations is sickle cell anemia, in which heterozygous individuals (Aa) have no or mild anemia and resistance to malaria infection while homozygous individuals (aa) with two copies of sickle cell allele have severe anemia and the homozygous individuals (AA) with two copies of the healthy allele are susceptible to malaria. Because of heterozygote advantage, all three genotypes are maintained in the population, due to balancing selection; thus sickle cell anemia is common in populations exposed to malaria despite its severe symptoms. However, it is unclear how common the phenomenon of overdominance is in general. Theoretical predictions suggest that a substantial fraction of beneficial mutations that arise in diploids will be overdominant, but to date few studies have systematically determined whether this is in fact the case. We are determining the prevalence of overdominance by testing adaptive mutations that arose during diploid evolutions, whose evolutionary progression was monitored using lineage tracking; we isolated many heterozygous adaptive mutants from the evolving populations. By generating all three genotypes (homozygous, heterozygous, and ancestor) of these adaptive mutations and measuring their fitness in a pool across many different conditions, we can compare their fitness, observe the frequency of overdominance, and test whether overdominance is pervasive across conditions. As an orthogonal approach to examining overdominance, we are analyzing data from the homozygous and heterozygous yeast deletion collection, to see if there is evidence of overdominance across many different conditions. This research will provide insight on the characteristics of overdominance and potentially how this phenomenon may have affected evolution and selection of various traits, including heritable diseases.

171 Centromere repositioning causes inversion of meiosis and generates a reproductive barrier

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For eukaryotic species, the chromosomal position of each centromere is distinct and epigenetically inherited with high fidelity, although the mechanisms underlying the epigenetic stability and its functional significance remain largely unknown. Here in the fission yeast Schizosaccharomyces pombe, we report that inner kinetochore impairment (e.g. single depletion of the conserved CENP-T-W-S-X complex subunits), upon going through meiosis, induces centromere repositioning - inactivation of the original centromere and formation of a new centromere nearby - in one of the three chromosomes at random. Repositioned centromeres reside in the pericentromeric heterochromatin regions asymmetrically, although heterochromatin is not required for inactivation of the original centromere or maintenance of the repositioned centromere. When cells carrying a repositioned centromere are crossed with those carrying the original centromere, the progeny suffers severe lethality due to defects in meiotic chromosome segregation. However, repositioned centromeres are competent in mitosis and homozygotic meiosis. Furthermore, homozygotic repositioned centromeres undergo meiosis in an inverted order, i.e., sister chromatids segregate first, and homologous chromosomes separate second, whereas the other original centromeres in the same cell undergo canonical meiosis. Together, these results recapitulate a reproductive barrier that could initiate genetic divergence between two populations with mismatched centromeres, documenting a potential role of the Evolutionary New Centromeres (ENCs) in speciation; and also, reveal the high flexibility and adaptation in the process of meiosis.

172 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 [2].

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173 Cytoplasmic age-dependent protein aggregates control DNA-circle accumulation in the aging mother cells in S.cerevisiae

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Aging is a progressive phenomenon observed in many organisms including the budding yeast Saccharomyces cerevisiae. S. cerevisiae divide asymmetrically resulting in the generation of a rejuvenated daughter budding off an aging mother. Protein aggregates and DNA-circles accumulating over time in the mother cell contribute to its aging, i.e. limits its lifespan. Accordingly, cells lacking the heat shock protein Hsp42 or overexpressing the metacaspase Mca1 fail to form a protein deposit with age and their lifespan is extended compared to wild type cells. Mother cells that do not accumulate age-dependent protein aggregates fail also to retain and accumulate DNA-circles. The defect in circle retention correlates with a defect in the formation of a strong nuclear diffusion barrier in the nuclear envelope. Importantly, the formation of a strong barrier was restored in the hsp42Δ mutant and Mca1 overexpressing cells upon the inactivation of Mks1, a protein involved in communication between the mitochondrion and the nucleus. Restoring the nuclear diffusion barrier strength, in cells lacking a cytoplasmic age-dependent protein aggregate abolishes their longevity phenotype. In light of our observations we propose that age-dependent protein aggregates cause aging by promoting the retention and accumulation of DNA-circles. Furthermore, our data suggest that mitochondria function as stress sensor for regulation of longevity.

174 Domestication Reprogrammed The Budding Yeast Life Cycle

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Domestication of plant and animal agricultural crops by selecting traits beneficial for human needs is the foundation for feeding the world population. At the same time, domestication imposes suites of detrimental side effects, syndromes, whose causes and effects are poorly understood, particularly for microorganisms. Here, we report that domestication of the baker's yeast completely reprogrammed its life cycle and changed the very foundation for its existence. We tracked reproduction, sporulation and chronological life span of quiescent cells across multiple environments across over 1000 isolates and we found a remarkably systematic dichotomy between domesticated and wild yeasts.

Firstly, domestication enhanced fermentative traits at the cost of respiratory traits and stress tolerance, except for stresses tightly linked to industrial environments.

Second, wild yeasts trigger meiosis and enter a resistant spore state in response to a wide range of nutrient depletions, domesticated yeasts have lost, or severely reduced, the capacity to enter this life cycle stage, instead remaining as quiescent cells. The loss occurred as independent events in domesticated clades across the phylogenetic tree. Survival in the quiescent stage was not affected by this life cycle change but remained independent of domestication and it is mainly dictated by population structure.

We further traced genes underlying the domestication reprogramming of the yeast life cycle, disclosing many of the genetic factors involved in the most dramatic event in recent yeast evolutionary history. Our results show that much of the knowledge extracted from one of our key model species reflect domestication rather than natural biology and may need to be re-evaluated in this light.

175 The Peroxiredoxin-Hsp70 Interplay Slowing Down Yeast Replicative Aging Deciphered by NMR, a Molecular Microscope.

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Peroxiredoxins (PRDXs) are thiol active peroxidases responsible for the regulation of peroxide levels in cells thanks to the presence of a peroxidatic cysteine within their active site. Upon elevated peroxide levels, they are subject to catalytic inactivation by hyperoxidation, which involves structural modifications and a switch to chaperone function1. Recently, the major cytosolic PRDX in S. cerevisiae, Tsa1, was shown to recruit the molecular chaperone Ssa1 to aggregated proteins through an H2O2-specific redox switch. In addition, normal Ssa1/2 levels are required for increased Tsa1 levels to slow down yeast replicative aging2. Interestingly, the sulfinylation of Tsa1 supports Ssa1 binding to damaged proteins accumulating during the aging process and its subsequent reduction triggers the disaggregation of misfolded and aggregated proteins2,3. The molecular switch between peroxidase and chaperone functions seems to be a key aspect of its function. However, besides the fact that Tsa1 and Ssa1 physically interact, the structural and dynamical details of this interaction at the molecular level, as well as the functional consequences for both enzymes, remain elusive.

Using advanced high-resolution NMR-spectroscopy, we are studying the Tsa1-Ssa1 interaction in vitro in order to characterize this complex in molecular detail and to decipher the functional consequences for both enzymes. We believe that elucidating this interplay can help understanding of the aging process and pave the way to find new targets for age-related diseases such as cancers. As classical solution state NMR-approaches are limited regarding the size of the system (300 kDa), we have used two alternative approaches. First, we characterize stable smaller Tsa1-variants using site directed mutagenesis. Second, by applying methyl-TROSY NMR experiments in combination with methyl-specific isotopic labeling to be able to study larger wild-type protein complex4. Finally, in the characterization of the chaperone Ssa1, we have implemented a divide-and-conquer approach to study both sub-domains of Ssa1 separately5. Here, we present the initial characterization of a decameric Tsa1-WT and Tsa1-S78D variant, forming mostly dimers, correlated with first NMR experiments on specific-methyl-labeled Tsa1, as well as preliminary NMR data obtained on Ssa1 subdomains.

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176 Developing DhNik1 as a crowding responsive stress sensor

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One of the grand challenges when using microorganisms as industrial production strains is to stably introduce the required orthogonal components.

The overexpression of a desired product can lead to a situation of increased macromolecular crowding (i.e. the volume fraction occupied by macromolecules within the cell) through accumulation and aggregation of proteins. The stress imposed on the cells can affect their viability.

In this project we aim to develop a crowding responsive stress sensor based on the well characterized S. cerevisiae High Osmolarity Glycerol (HOG) pathway and the Debaryomyces hansenii group III hybrid histidine kinase (HHK) DhNik1.

DhNik1 has been shown to rescue and functionally complement the otherwise lethal deletion of the group IV HHK SLN1 gene. HHKs involved in the sensing of osmolarity stress are usually situated in the membrane, where they are hypothesized to monitor membrane curvature and external salt concentrations. However, group III HHKs are devoid of membrane interacting domains. We therefore hypothesize, that the physical stimulus for DhNik1 is likely to be the macromolecular crowding effect caused by the efflux of water molecules upon hyperosmotic upshift.

We will further investigate the activating stimulus of DhNik1 and develop a sensory pathway to monitor macromolecular crowding in industrial production strains. Further, we want to employ a similar pathway to modulate expression of an orthogonal pathway in a crowding responsive inhibition feedback pathway. We anticipate this to be a useful tool to optimize production efficiency, through minimization of detrimental side effects imposed on individual cells by the orthogonal expression.

177 Systematic analysis of bypass suppressors of essential genes

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The functional consequence of a mutation can depend upon the genetic background in which it occurs. In the most extreme case, a gene can be essential in one genetic background, but have no effect on cell growth in another. To systematically identify the genetic factors that affect context-dependent gene essentiality, we examined 728 essential genes for rare cases in which a spontaneous suppressor mutation could overcome the lethality associated with a deletion allele of the essential gene. In total, we discovered 124 essential genes (~17%) that showed context-dependent essentiality, and identified the genetic alterations driving the suppression. These dispensable essential genes frequently played a role in transport or signaling processes and tended to be less well conserved across species than other essential genes. Although for most essential complexes, either all or none of the subunits were dispensable, in a few cases only loss of specific submodules of the complex could be compensated for. The suppressor mutations commonly involved aneuploidies or gain-of-function events in genes that were essential themselves. When multiple independent suppressor genes were identified for a query gene, they often encoded members of the same pathway or complex. In summary, this work produced a validated resource of the drivers of context-dependent essentiality, revealing how the rewiring of cellular processes can tolerate the deletion of genes that are normally indispensable for cellular viability.

178 Employing Artificial Promoters in Yeasts Using the Protein Expression Platform GeneEE.

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Yeasts are one of the core organisms that are utilized for production of proteins on an industrial scale. They are easy to grow and manipulate and can deliver secreted protein expressions in grams per liter. Unfortunately, not every attempt at protein production with yeasts is successful. Many factors play a role in the multiple step procedure that leads from a gene to a protein. One of the factors is the interplay between the driver of gene expression, the promoter, and the gene of interest. The mechanisms of this interplay are not fully understood yet, leading to many unsolved questions resolving around failed protein expression. GeneEE is a protein expression platform that attacks this problem. The platform employs a library of artificial promoters that can be inserted upstream of a gene. This leads to a wide range of expression profiles of the gene. Clones of the needed expression strength can be picked and used for protein production. GeneEE has been successfully applied in different organisms, among them Saccharomyces cerevisiae and is currently being tested in Pichia Pastoris.

179 Species-specific Response of Telomeres to Stress

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Telomeres are nucleo-protein structures at the ends of linear chromosomes whose maintenance is essential for the protection of genome integrity. The length of telomeric arrays sensitively respond to various environmental factors. For example, in baker's yeast Saccharomyces cerevisiae, high concentrations of ethanol cause telomere elongation. In this work, we performed a comparative analysis of the effect of ethanol on the telomere maintenance in different yeast species. Upon ethanol treatment, we observed telomere elongation both in S. cerevisiae and fission yeast Schizosaccharomyces pombe, whereas the treatment had no effect on telomere length in other species such as Yarrowia lipolytica and Candida parapsilosis. Formaldehyde (FA) is another toxic substance, often used for DNA and protein crosslinking, while also being produced endogenously. It is metabolized by FA dehydrogenase encoded by the SFA1 gene in S. cerevisiae. We investigated the effect of FA on telomeres in the S. cerevisiae wild-type and sfa1Δ mutant cells and found that their length remained unchanged in both analyzed strains. Finally, we have found that the Yarrowia lipolytica terΔ strain lacking functional RNA subunit of telomerase overexpresses several genes involved in the oxidative stress response, including DDR48. In contrast, the levels of Ddr48 protein in tlc1Δ strain of S. cerevisiae are comparable with the wild-type strain and it does not change in later generations. These results demonstrate that telomeres respond react to intra- and extracellular stress in a species-specific manner and thus the effect of stress factors should be analyzed individually in different species.

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180 Runaway Evolution of Yeast Telomeric Repeats: In Search of the First Steps

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Among other eukaryotes, tracing back the evolutionary history of yeast telomeric repeats and telomere-binding proteins is particularly complicated, since both of these features exhibit only limited similarities to their counterparts in other lineages. In most yeast species, telomeric repeats are relatively long (up to 26 bp), not always G-rich and sometimes heterogenous. In addition, telomeric sequences in different yeast species are recognized by several types of DNA-binding proteins interacting with various protein partners resulting in an unprecedented diversity in telomere-maintenance system. In order to inspect the evolutionary processes leading to this diversity in more detail, we analyzed the DNA-binding properties of Tay1p, a telomere-binding protein of Yarrowia lipolytica, a yeast species whose telomeric repeat resembles the common eukaryotic motif 5'-TTAGGG-3'. Specifically, its telomeric repeat is composed of this motif disrupted by a four nucleotide long spacer (5'-TTagtcAGGG-3'). Using the oligonucleotides with telomeric sequence of several closely related yeast species as a substrate, we showed that presence of the 5'-TTAGGG-3' motif is crucial for Tay1p binding, while the changes in spacer element are tolerated. Moreover, a specific arrangement of the nucleotides in spacer region might significantly increase the affinity of Tay1p, suggesting an array of 2-3 guanine nucleotides is a key feature of the repeat, responsible for high-affinity binding in vitro. To further analyze the evolutionary consequences of the diversification of telomeric repeats, we used the telomeric sequences of evolutionarily more distant species as the substrate for Tay1p and designed a series of probes with variants of either 5'-TTAGGG-3' motif or the spacer element to test the ability of the protein to recognize the cognate sequence. Altogether, our data suggest that the insertion of spacer element into the ancient telomeric repeat might have been one of the initial steps leading towards diversification of yeast telomeric repeats, and as the diversification progressed, the evolutionarily ancient telomeric proteins (such as Tay1p) lost the ability to bind telomeric sequences with sufficient affinity and/or flexibility. Inevitably, at certain point these proteins lost their function at telomeres and were replaced by new and possibly more flexible telomere-binding proteins.

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181 Cross-talk between pH Homeostasis and Vacuolar Amino Acid Metabolism: an Essential Aspect of Ageing?

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During the past decades, significant progress has been made in unraveling the dynamic, but tightly regulated, nutritional responses in yeast, resulting in the identification of signalling routes that formed the basis of more complex nutritional and hormonal pathways in higher multicellular eukaryotes. Some of these conserved pathways impact on several aspects of cellular ageing. Given the close interaction between nutrient availability, signalling and pH homeostasis, it is not surprising that pH could also be linked with processes involved in cellular homeostasis and ageing. We recently reported a correlation between vacuolar acidity and chronological lifespan, while others made similar observations for replicative lifespan. Hence, a better understanding of how intracellular pH is controlled will aid to further unravel the complex process of ageing. While the connection between carbon metabolism and pH homeostasis has been extensively investigated, much less is known about the importance of nitrogen metabolism. Our recent findings suggest a crosstalk between vacuolar amino acid availability and vacuolar pH homeostasis. This close interaction between nitrogen metabolism and pH homeostasis is not surprising, as vacuolar pH will be important for autophagy and the functioning of several amino acid transporters at the vacuolar membrane. We are now further looking into the possibility that pH-dependent vacuolar amino acid storage is a crucial factor for chronological lifespan determination, as this was already implied for replicative lifespan.

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182 Cationic Stress Response of Yeasts

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Sensitivity to salts is one of the most common stress factors faced by microorganisms in industrial fermentations. In part, it is due to the presence of cations (like Na+, sodium and K+, potassium) in cultivation environment. Cationic balance can significantly affect the efficiency of bioprocesses and survival of microorganisms, making it a relevant topic to study. Currently, both cations, Na+ and K+, have been independently studied to establish their roles in cellular physiology. However, the effect of these two cations on cellular physiology together are not extensively investigated. Our study fills this knowledge gap and investigates interaction effects of these two cations in industrially relevant yeasts.

Potassium ions are reported to be essential for Na+ stress tolerance and mutations increasing its intracellular concentration are reported to improve stress tolerance in yeast (Gaxiola R. et al., 1992). Latter prompted us to inquire whether an increase in potassium ions in culture can lead to an increase in Na+ stress tolerance. We supplemented a minimal glucose-based medium with combinations of various K+ and Na+ concentrations and used Saccharomyces cerevisiae (CEN.PK-7D and W303), Kluyveromyces marxianus (CBS6556) and Rhodotorula toruloides (CCT0783) for evaluation of dual cation stress responses.

One of our key findings showed that a decrease in growth rate caused by Na+ is partially rescued by supplementing K+ in a culture of S. cerevisiae (CEN.PK-7D), but do not affect other yeasts. Moreover, the ability of K+ to rescue growth rate shows a dependency on a carbon source. Cellular volume was evaluated both at a single cell and population levels and it was found that the change of the cellular volume after the stress response and the recovery time play an important role for the salt sensitivity and cellular fitness. Information about the strain-dependent responses of salt stress will allow us to speculate the stress response mechanisms and validate these theories experimentally.

183 The Mut+ Strain of Komagataella phaffii (Pichia pastoris) Expresses PAOX1 5 and 10 Times Faster Than Muts and Mut− Strains: Evidence That Formaldehyde or/and Formate Are True Inducers of AOX.

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The methylotrophic yeast Komagataella phaffii is among the most popular hosts for recombinant protein synthesis. Most recombinant proteins were expressed in the wild-type Mut+ host strain from the methanol-inducible promoter PAOX1. Since methanol metabolism has undesirable consequences, two additional host strains, Muts (AOX1-) and Mut− (AOX1- AOX2-), were introduced which consume less methanol and reportedly also express recombinant protein better than Mut+. Both results follow from a simple model based on two widespread assumptions, namely methanol is transported by diffusion and the sole inducer of PAOX1. To test this model, we studied 14C-methanol uptake in the Mut− strain and β-galactosidase expression in all three strains. We confirmed that methanol is transported by diffusion, but in contrast to the literature, Mut+ expressed β-galactosidase 5- and 10-fold faster than Muts and Mut−. These results imply that methanol is not the sole inducer of PAOX1 — metabolites downstream of methanol also induce PAOX1. We find that formate or/and formaldehyde are probably true inducers since both induce PAOX1 expression in Mut− which cannot synthesize intracellular methanol from formate or formaldehyde. Formate offers a promising substitute for methanol since it does not appear to suffer from the deficiencies that afflict methanol.

184 Robustness of nutrient signalling is maintained by interconnectivity between signal transduction pathways

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Crosstalk in biology is any phenomenon by which an intracellular signal transmitted in one signalling pathway creates an effect in another pathway. In nutrient signalling pathways crosstalk has an important function. Typically, to study these pathway interconnections perturbation of the signalling system is involved. Unfortunately, perturbations often produce noise which causes a major challenge in identification of interconnections. Employing systems biology approach by developing appropriate models can provide minimal intervention in these systems. Signalling pathways are usually modelled by ordinary differential equations, however, creating dynamical models of signalling pathways of a realistic size is still an obstacle. To overcome this problem, we developed a vector-based reaction-contingency Boolean logic model of the yeast Saccharomyces cerevisiae nutrient sensing pathways cAMP-PKA, Snf1, Snf3-Rgt2 and TOR pathway. To increase the information content of Boolean model from simple 'active' and 'inactive', we assigned a vector to each component describing following features: localization, phosphorylation status, guanylation status and DNA binding status. This model approach is highly modular and easy to expand to other signalling pathways. We further found during the gap filling process that most lacking components are phosphatases which exposes a lack of knowledge on phosphatases involved in the sensing process. The gap filling process also identified crosstalk from the PKA and Snf1 pathway to other pathways as a vital aspect to make the model switch between nutrient conditions. We simulated the model with known crosstalk combinations. Subsequent analysis of the simulations that the crosstalk from the Snf1 pathway to the Rgt2/Snf3 pathway contributes to the robustness of this signalling network. Altogether, this work shows that network interconnections lead to the robustness of signalling pathways. Our approach contributes to the understanding of the function and importance of crosstalk in nutrient signalling.

185 Yeast Colony Differentiation and Long Non-coding RNA.

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The formation of differentiated cell types as yeast colonies age is regulated at the transcriptomic and translational levels [1,2] and possibly by epigenetic mechanisms and long non-coding RNA (lncRNA). We recently identified [3, 4, 5] differences in the expression of genes and associated lncRNA in surface and sub-surface parts of wild-strain, biofilm colonies, as well as those in subpopulations of laboratory strain smooth colonies, occuring at different developmental stages . We showed that converse regulation of, in particular, antisense-overlapping lncRNA may temper the expression of "target" genes with key cellular roles, ranging from cell cycle control and cell wall integrity to amino acid biosynthesis and transport. This work was supported by GACR 19-11384S and LQ1604 NPU II. Attendance at this conference is supported by MiCoBion.

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186 Mitochondrial Retrograde Signaling and its Role in Yeast Colony Development

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A yeast colony is a highly organized structure, in which cells differentiate and form various subpopulations. Previously, we have described differentiation of smooth colonies of Saccharomyces cerevisiae laboratory strains into two major subpopulations of cells. These cell subpopulations are spatially separated and have different physiological, morphological and metabolic characteristics. Some similarities between the processes typical of differentiated yeast colony cells and those associated with tumour development were also described [1]. More recently, we have identified important role of mitochondrial retrograde signalling (RTG) in colony development. We showed that RTG pathway regulation is complex and contains three distinct branches, which are specific for different cell subpopulations and probably regulated by signals from differently adapted mitochondria [2]. We have now acquired data supporting a role of mitochondria in regulation of one specific branch of RTG signalling and have partially described a mechanism of this regulation (not published). The obtained data contribute to revealing the regulatory role of mitochondria in the formation of yeast colonies. The project is supported by the Grant Agency of the Czech Republic (19-09381S).

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187 The Hsp70 Homolog Ssb: a novel Component of the TORC1/Sch9 Signaling Network.

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The Hsp70 chaperone Ssb and its cochaperone RAC serve a dual role in protein synthesis. First, Ssb/RAC bind to the ribosome directly and together facilitate the folding of newly synthesized polypeptide chains (1,2). Second, Ssb/RAC are required for ribosome biogenesis, such that in the absence of the chaperone system yeast cells contain a reduced number of ribosomes, display enhanced aggregation of ribosomal particles, and display subtle structural changes within core ribosomal particles, which manifest in reduced translational fidelity (2,3).

We found that the role of Ssb/RAC in ribosome biogenesis is causally linked to a moonlighting function of Ssb/RAC in the regulation of the TORC1/Sch9 signaling pathway (4). The AGC family kinase Sch9 is required to warrant sufficing ribosome production in fast growing yeast cells (5,6). Ssb is a posttranslational component of the TORC1/Sch9 signaling pathway and is required for the proper TORC1-dependent phosphorylation of Sch9 at T737, which activates Sch9, and turns on ribosome biogenesis. Our data reveal that ribosome aggregation is not confined to cells lacking Ssb but also occurs in cells lacking Sch9, indicating that Sch9 activity is required to prevent ribosome aggregation. Moreover, a permanently active Sch9 mutant partially suppresses ribosome aggregation in cells lacking Ssb (4). The combined findings reveal that Ssb/RAC does not only act via its general foldase activity towards newly synthesized polypeptides, but also posttranslationally to ensure proper signaling via the TORC1-Sch9 pathway.

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188 Fungal Fludioxonil Sensitivity is Relieved by Overexpression of CORVET Complex Components

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Fludioxonil belongs to the phenylpyrrole class of antifungal compounds and its molecular target is Hybrid Histidine Kinase 3 (HHK3), a conserved cytosolic osmosensor found in higher yeasts and fungi. Since HHK3 causes activation of p38/Hog1 MAPK in the presence of fludioxonil, abnormal accumulation of glycerol, (which is otherwise a natural response to osmostress), and subsequent cell lysis was presumed to be the cause of fludioxonil sensitivity. To examine the use of fludioxonil against human fungal infections, its mode of action was studied in S. cerevisiae, which loses its resistance to fludioxonil in presence of heterologous HHK3.

Using Confocal microscopy, we showed that unlike osmostress, phosphorylated Hog1-GFP did not translocate to the nucleus in the presence of fludioxonil. Further, we found that fludioxonil sensitivity persisted even when Hog1 translocation to the nucleus was blocked. Fludioxonil sensitivity remained unaffected even when glycerol biosynthesis pathway was blocked in S. cerevisiae. Thus, we ruled out glycerol accumulation to be the primary cause of fludioxonil toxicity.

A systematic screen of HHK3 transformed haploid deletion strain library of S. cerevisiae at a subthreshold dose of fludioxonil identified 60 deletion strains hypersensitive to fludioxonil. Class C core vacuole/ endosome tethering (CORVET) complex was the most overrepresented protein complex identified in fludioxonil hypersensitive profile. Additionally, 30% of the genes were involved in the establishment of localization of proteins and >6% of the genes were related to cell wall biogenesis, cytokinesis, and pseudohyphal growth. Identified genes were over-expressed and their resistance checked towards fludioxonil to identify genes directly related to its mode of action. Overexpression of only CORVET components could alleviate fludioxonil toxicity in S. cerevisiae model and Candida albicans. Through confocal microscopy of GFP-tagged CORVET components, we found that impaired localization of CORVET complex was the primary cause of fludioxonil toxicity as major dependent cellular processes like endosomal transport, vacuole organization, cytokinesis, and hyphal growth were affected. Overexpression of CORVET components could restore all affected processes in the presence of fludioxonil in S. cerevisiae model as well as C. albicans. These results clearly demonstrated that fludioxonil impedes endosomal trafficking by interfering with CORVET localization downstream to HHK3.

189 The Well-Tempered Controller-846: A Precisely Titratable and Tight Transcriptional Controller in S. cerevisiae.

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Conditional gene expression is central to biological experimentation and discovery. A common approach is using inducible transcriptional controllers that determine protein levels based on an input. In S. cerevisiae, the most commonly used ones rely on either a metabolite relieving repression of a transcriptional activator (TA) (e.g. PGal1-galactose system) or a TA fused to a DNA binding domain that can be activated by a small molecule like β-estradiol. However, the former limits the usable media conditions, and the latter relies on TAs that can be toxic. Furthermore, most systems have a switch-like, on/off behaviour leading to high heterogeneity at intermediate expression levels. This makes population level measurements difficult to interpret. Last, presence of basal leakiness and low maximum expression mean that protein dosage phenotypes of some essential genes cannot be studied.

Here we present the Well-tempered Controller-846 (WTC846), which overcomes all limitations of existing controllers. It is based on the strong constitutive TDH3 promoter active under many growth conditions. We engineered it such that the bacterial repressor TetR competes with the transcriptional machinery for binding. Induction of transcription therefore relies solely on removal of TetR using anhydrotetracycline. We then created a negative feedback loop by placing TetR under the control of this new promoter. This led to an extended titratable range and homogenous protein levels at all doses. Last, we abolished basal expression by low, constitutive expression of an engineered TetR-Tup1 molecule. Overall WTC846 combines eukaryotic and prokaryotic elements for precise titration of a large range of protein levels without basal leakiness.

Placing low expressed, stable and instable proteins like Cdc28 and Ipl1, as well as abundant proteins like the glycolytic enzyme Tpi1 under the control of WTC846 recreated the known knock out and overexpression phenotypes. We synchronized an entire batch culture by controlling Cdc20 expression with WTC846 and demonstrated fast and homogenous release of the population. Importantly, we titrated Tor2, Pbr1 and Pma1 to not only observe distinct, protein dosage dependent growth rates but also morphological changes with a high penetrance of the phenotype specific to different growth conditions. Overall, we show that WTC846 allows for dynamic and precise control of protein dosage under various experimental conditions without affecting cell physiology.

190 Diffusion and Localization of Proteins in the Plasma Membrane of Saccharomyces cerevisiae.

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The plasma membrane (PM) of Saccharomyces cerevisiae contains membrane compartments, MCC/eisosomes and MCPs, named after the protein residents Can1 and Pma1, respectively. Using high-resolution fluorescence microscopy techniques we show that the amino acid transporters Can1 and Lyp1 are able to diffuse into the MCC/eisosomes, where a limited number of proteins are conditionally trapped at the (outer) edge of the compartment. Our data indicate that the mobile fraction of all integral plasma membrane proteins tested shows extremely slow Brownian diffusion through most of the PM; the diffusion coefficients are about 3-orders of magnitude slower than typically found for proteins in the PM of bacteria and mammalian cells. We also show that proteins with large cytoplasmic domains, such as Pma1 and synthetic chimera of Can1 and Lyp1, are excluded from the MCC/eisosomes. Additionally, we investigated the lateral diffusion of proteins in the PM as a function of temperature. We find that the lateral diffusion coefficient increases about 7-fold when the ambient temperature is increased from 25 to 50oC, a change in mobility that is entirely reversible. We also find that mobility of proteins in the plasma membrane yeast is higher when cells are grown at lower temperatures and subsequently analyzed at higher temperatures and vice versa. We conclude that the intrinsic properties of the lipids rather than compartmentalization of the yeast PM are responsible for the slow diffusion of integral membrane proteins.

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191 Young Genes are More Responsive to Environmental Stress than Ancient Genes in Budding Yeasts.

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Members of the subphylum Saccharomycotina (budding yeasts) are found in many of Earth's biomes, and include industrially-relevant species like Saccharomyces cerevisiae, Kluyveromyces marxianus, and Yarrowia lipolytica. These species are attractive for industrial production of biomolecules, since they grow rapidly, utilize inexpensive growth substrates, and are readily engineered to produce heterologous products. However, one complication for production with these species is that ideal growth conditions may not match industrial reaction conditions, resulting in stress, slower cellular growth, and diminished product yields. These non-ideal conditions are difficult to eliminate from processes as they originate from feedstocks (osmolarity or pH), substrate processing (elevated temperatures), and products (product toxicity).

This work investigated the aforementioned yeast species after adaptation to low pH, high osmolarity, or high temperature to assess the commonalities between organisms after exposure to the same stress. This analysis found zero significant gene expression or protein abundance changes that occurred in all organisms in response to the same stress, despite analysis of 2,903 orthologous genes. Further analysis determined that the 2,903 shared genes were significantly unenriched for stress-responsiveness for each stress. Could this trend be influenced by the difference in gene age between orthologous genes (>400 million years) and non-orthologous genes (<400 million years)? To test this, genes were grouped by evolutionary age based on ortholog presence amongst diverse eukaryotes. This analysis found that each stress-response in each organism showed significant enrichment for the youngest gene group (species-exclusive genes) compared to ancient gene groups. Further, young genes were shown to sample twice as many amino acid sequence alterations per million years as ancient genes, suggesting that they adapt more rapidly.

Could young genes harbor adaptations that are found only in specific budding yeast species, similar to young genes in other organisms (e.g. antifreeze proteins in artic fishes)? Might the stress tolerances that have emerged during recent evolutionary time, like the temperature tolerance in K. marxianus or ethanol tolerance in S. cerevisiae, be influenced by young genes? While further work is needed, our multi-species multi-stress analysis suggests that to engineer stress tolerance, scientists may benefit from considering gene age.

192 Role of the ScMep2 Ammonium Transport Protein 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 (Gimeno et al., 1992). The transmembrane transport of ammonium (hereafter referring to NH4+ + NH3) is mediated by proteins of the conserved Mep-Amt-Rh family including the human Rhesus factors. S. cerevisiae possesses three members of this family (Mep1-3) (Marini et al., 1997). The activity of Mep1/3 and Mep2 is controlled by TORC1 and its effector kinase Npr1 via distinct mechanisms (Boeckstaens et al., 2015; Boeckstaens et al., 2014). 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 (Lorenz et al., 1998). However, the precise molecular mechanism of Mep2-mediated signal transduction remains unclear. We will present our data sustaining a close link between ammonium transport efficiency of Mep2 and its capacity to allow filamentation. Our data show that while the C-terminal domain of Mep2 contributes to maximal transport and signaling efficiencies, it is not absolutely required for conveying the signal of filamentation. Hence, filamentation signaling appears to occur in the absence of exclusive binding of partners to the Mep2 C-terminus. Our data further support that a conformational change accompanying substrate translocation in the hydrophobic core of Mep2 is required for the signaling property. Finally, using functional characterization in Xenopus oocytes, we show that substrate translocation via Mep1 and Mep2 operates via distinct mechanisms. Our data reveal a link between the substrate transport mechanism specific to Mep2 and its capacity to induce the filamentation signal.

193 Genome-wide imaging-based screening of protein phase separation under oxidative stress

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It is belived that liquid-liquid phase separation (LLPS) driven by mutivalent macromolecular interactions is an important organizing principle based on which many membrane-less compartments form through self-assembly of proteins and nucleic acids. And recent studies also highlight that non-membranes orangelle can function as a kind of seed to form pathological insoluble aggregates that associated with diseases. Hence, screening new proteins that can form phase separation could provide a novel clue for assembly mechanism of these biomolecular condensates and pathomechanisms of diseases. Due to higher ROS level in cells might be toxic and have thought to be a hallmark of age-asscoiated neurodegenerative diseases, so in this work we use several oxidative stress inducers (e.g. H2O2, paraquat and sodium arsenite), combined with Saccharomyces cerevisiae non-essential single deletion collection and high-content microscope, for performing genomic-wide screens of new proteins forming phase separation under oxidative stress. We have already explored the optimal paraquat screening condition and finished the first step of screening proteins that form phase separation under these conditions. Now we are analyzing the data and selecting potential proteins that can form foci. And next we will confirm phase separeted properties and functions of these hits by other biochemical experiments and reveal their relevance with human diseases.

194 Short-flank PCR-based gene targeting in lager yeast based on RAD51 overexpression

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Saccharomyces cerevisiae is well-known for its extremely efficient homologous recombination (HR) that enables PCR-based gene targeting with short-flanking homology regions of 45 bp to the target locus. This has been used industrially, for example, to generate the complete gene deletion collection. In lager yeast, a hybrid between S. cerevisiae and S. eubayanus, however, this efficiency of HR is drastically reduced. In our efforts to improve gene-editing in lager yeast we overexpressed ScRAD51, using the Saccharomycopsis schoenii TEF1 promoter, on a CEN/ARS-plasmid in the lager yeast strain Weihenstephan 34/70. This single alteration lead to a decisive improvement of successful gene targeting with 50 bp short guide sequences. We demonstrate this by targeting three open reading frames of three genes of the S. cerevisiae parental genome located on different lager yeast chromosomes: ADE2, HSP104, LSP1. PCR products used for transformation were derived from the kanMX6 marker and allowing for selection of transformants with G418. Additionally, hygromycin selection was used for maintenance of the RAD51 plasmid. Diagnostic PCR confirmed that successful gene targeting in lager yeast with short flanking homology regions depended on RAD51 overexpression. Further improvements in lager yeast transformation included optimized transformation protocols based on the lithium acetate and electroporation methods. The RAD51 plasmid can readily be lost upon growth under non-selective conditions enabling scarless strain alterations. These improvements open new possibilities in gene-function analyses of lager yeasts.

195 Isolation and Identification of a Novel Class of Oncostatic Glucose Transport Inhibitors Using the Yeast tps1Δ Mutant.

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The yeast Saccharomyces cerevisiae has been widely used as a model organism for the study of central metabolism. Like aggressive glycolytic cancer cells, yeast prefers fermentation of glucose over respiration. Many cancer cells display the Warburg effect: overactive flux through glycolysis, which is essential for their energy production and proliferation. The Trehalose-6-phosphate synthase 1 deletion mutant (tps1Δ) shows a behavior comparable to the Warburg effect, but with an even stronger glucose influx. This mutant can no longer produce trehalose-6-phosphate which inhibits hexokinase in yeast. When this mutant is given glucose, unbridled hexokinase activity causes upper glycolytic metabolites to accumulate until it dies because of Fru1,6bisP/Ras-mediated apoptosis (Peeters, Van Leemputte et al. 2017).

We have used tps1Δ to screen for small compounds that restore growth on 5 mM glucose. From 40,000 screened compounds, one positive hit, compound A, was identified. Mode-of-action studies revealed that it is a competitive glucose transport inhibitor that rescues the tps1Δ mutant by reducing glucose uptake, which normalizes the metabolite profile. Moreover, compound A inhibits transport activity of mammalian GluT1 expressed in the yeast hxt null strain, suggesting a conserved mode of action. In addition, compound A inhibits growth of the adenocarcinomic lung cancer cell line A549 in a concentration-dependent manner with reducing potency at increasing glucose levels. Structural analogs are being screened for activity in yeast and cancer cells. The new glucose transport inhibitor class may have unique potential for counteracting the Warburg effect, by restricting only hyperactive glucose metabolism in cancer cells and not basal glucose metabolism in normal human cells.

196 The Hsp70 homolog Ssb: moonlighting in metabolic signaling regulation

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SSB1 and SSB2 encode for two nearly identical homologs of the Hsp70 family chaperones, collectively called Ssb. Ssb binds to the ribosome via its C-terminal domain and interacts with a variety of nascent polypeptides to prevent their aggregation and assist de novo folding.

Besides its classical Hsp70 foldase activity Ssb serves specific posttranslational functions, which are connected to the regulation of metabolic signaling kinases. For instance, Ssb interacts with and is involved to the regulation of the kinase Sch9, a direct target of TORC1, which is required for the regulation of ribosome biogenesis and translation (1). Furthermore, Ssb is also closely intertwined with the SNF1 signaling pathway (2,3). SNF1 is the ortholog of the mammalian AMP-activated kinase (AMPK) and is a key component of the response to glucose starvation in Saccharomyces cerevisiae (4). The heterotrimeric SNF1 complex is activated by phosphorylation of the alpha-subunit Snf1 at T210. When active, Snf1 phosphorylates its downstream targets, including transcriptional regulators, which results in the derepression of glucose repressed genes. Snf1 is dephosphorylated by the PP1-type phosphatase Glc7 and its regulatory subunit Reg1. Deletion of REG1 results in hyperphosphorylation of Snf1-T210, derepression of glucose repressed genes, and severe growth defects.

Our data reveal that Ssb is involved in the regulation of SNF1 activity. Similar to the deletion of REG1, deletion of SSB1/SSB2 results in transcriptional derepression of glucose repressed genes. Ssb is in close, posttranslational contact with several members of the network regulating SNF1 activity. Moreover, overexpression of Ssb1 in a reg1 deletion strain abolishes Snf1-T210 hyperphosphorylation and associated growth defects. We find that Ssb1 interacts with the 14-3-3 proteins Bmh1/Bmh2 via the same C-terminal region of Ssb that tethers Ssb1 to the Reg1 complex and is required for ribosome binding. Furthermore, overexpression of Bmh1, like overexpression of Ssb1, complements the severe growth defects of a reg1 deletion strain. Based on these results we suggest a model in which Ssb and Bmh combine their unique client binding properties to form a novel chaperone module, which is involved in the regulation of SNF1 activity.

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197 Oscillatory Behaviors in Snf1 Signaling Network.

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To maintain cellular homeostasis the cell needs to be able to detect a myriad of situations and adapt the cellular biochemical machinery accordingly. The detection, transmission and the appropriate response of the cell towards the numerous stimuli is performed by a network of signal transduction pathways. Therefore, it is vital for the cell to have a reliable and fast response of the proper signal pathway in response to a specific network. Failure of signal-transduction pathways can lead to pathological conditions such as cancer or diabetes. The traditional model of latent transcription factors includes activation and subsequent relocation. However, it has been shown that some transcription factors, like Mig1 and Mig2, are activated in a pulsatile manner during static conditions1. Both of those transcription factors are involved in glucose repression and are controlled through the Snf1 pathway. In this work we set out to understand whether the oscillatory behavior is a prevalent behavior in the Snf1 pathway. We will characterize a number of Snf1 downstream targets after short- and long-term exposure to different concentrations of glucose. We will examine if SNF1 and its sub-units also shows oscillatory behavior. This work will contribute to the understanding of the pulsatile behavior, its role in glucose signaling and help elucidate how failure in control can lead to pathological conditions.

198 Absolute Quantification of the Mitochondrial Proteome Reveals the Dual Role of Mitochondria in Diauxic growth of Saccharomyces cerevisiae.

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Being Crabtree-positive, S. cerevisiae has evolved the ability to ferment glucose through the glycolytic pathway, as well as to metabolize the ethanol produced during fermentation, through respiratory pathways occurring in mitochondria. The transition phase from fermentative to respiratory metabolism, known as the diauxic shift, is reflected by dramatic rearrangements of mitochondrial function and structure. To date, however, subcellular quantitation of mitochondrial metabolism during the diauxic shift is lacking. We performed bioreactor batch cultivations of S. cerevisiae and quantified the absolute proteome of cells and isolated mitochondria, harvested during fermentative growth on glucose, the diauxic shift and respiratory growth on ethanol. Furthermore, we engineered the yeast to constitutively express two fluorescent proteins, GFP and mCherry, bearing mitochondrial targeting sequences for mitochondrial import, thereby enabling a precise deduction of the biophysical properties (i.e. volume and mass) of the mitochondrial network using state-of-the-art optical imaging techniques. This allowed, for the first time, the determination of absolute protein abundances at a subcellular level. Our data shows that the transition from fermentative to respiratory metabolism triggers a reallocation of the mitochondrial proteome from biosynthetic to energy-related processes as well as a drastic increase in the size of the mitochondrial network. By tracking the transformation of mitochondrial morphology, alongside changes occurring in mitochondrial proteome allocation, our research paves the way for a deeper understanding of mitochondria, in particular how mitochondria balance their role as a biosynthetic hub as well as a center for cellular respiration.

199 Overexpression Of Citrate Synthase Increases Isocitric Acid Biosynthesis In The Yeast Yarrowia Lipolytica.

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The non-conventional yeast Yarrowia lipolytica, known for its ability to accumulate large amounts of lipids is also an excellent citric (CA) and isocitric acids (ICA) producer. This feature combined with its abilities to use wide spectrum of substrates, such as n-alkanes, oils, hydrolyzed molasses and glycerol makes CA production by Y. lipolytica an interesting alternative to processes with A. niger.

In this study Y. lipolytica strains derived from a CA overproducing A101.1.31 UV mutant with modifications in citrate synthase genes (CIT) were constructed and tested for citrate synthase activity and abilities to produce CA and ICA from glycerol and rapeseed oil (bioreactor study).

The results showed, that over expression of either citrate synthase genes (CIT1 and CIT2) enhanced citrate synthase activity, respectively by a factor of 4 and 6. Interestingly, the strain with disrupted CIT1 was unable to use propionate as a sole carbon source what proved that CIT1 gene encodes a protein with dual activity – citrate and 2-methylcitrate synthase. In the overexpressing mutants, biosynthesis of citric acids increased in noticeable trend towards ICA production in both glycerol and rapeseed oil media. CIT1 and CIT2 strains produced CA and ICA from vegetable oil in a ratio close to 1, respectively 67.8/65.4 and 53.6/56.2 (in g/L).

The present study shows that Y.lipolytica could be used as a platform for efficient production of isocitric acid, a valuable substrate for chiral syntheses in pharmaceutical industry.

200 A Theoretical Approach to Understand the Role of the Retention Mechanism in the Rejuvenation Process

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During yeast cell division damaged proteins are inherited asymmetrically such that most are retained within the mother cell, resulting in an ageing mother and a daughter cell with full replicative potential. However, daughters of old mothers are born with increasing levels of damage resulting in lower replicative potential. Remarkably, these prematurely old daughters can nevertheless give rise to rejuvenated cells with low damage levels and full replicative potential. The mechanisms of how aged cells give rise to young progeny are however not completely understood. We have developed a computational framework for replicative ageing to elucidate the role of damage retention in the rejuvenation process, on both a single cell and population level. We further investigate the link between the repair mechanism and rejuvenation. The proposed model is capable of simulating complete cell lineages and can explicitly track mother-daughter relations in dynamically growing yeast populations. Accounting for the individuality of the cells, we extract model parameters from single cell growth data generated in a microfluidics device. Our approach suggests that a damage retention mechanism is favorable for asymmetrically dividing organisms, such as S. cerevisiae, as they lead to more resilient populations less prone to environmental stress and parameter variations. Further, by analyzing the full pedigree tree, more detailed information about the damage distribution between generations and siblings will be gained, allowing us to find parameters and conditions that correlate to rejuvenation. With large-scale explorations of population properties and by following individual cells in this setting, we hope to shed light upon rejuvenation as an effect of damage retention in yeast.

201 The yeast Vsb1 and Ypq2 transporters coordinate import and export of vacuolar arginine

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Yeast vacuoles share many properties with the lysosomes of animal cells and fulfill multiple roles including nutrient storage. A typical response of yeast cells faced to starvation for a specific nutrient is mobilization to the cytosol of replenishing compounds stored in their vacuole. However, how the activity of vacuolar transporters catalyzing nutrient import and export is coordinated remains unknown. We have now characterized Vsb1 (Vacuolar transporter for Storage of Basic amino acids 1) as a novel vacuolar transporter essential to arginine accumulation into the vacuole. We show that Vsb1 is active in cells growing under nitrogen replete conditions where it is required for H+-dependent arginine uptake into the vacuole. Under nitrogen starvation conditions, the vacuolar arginine stocks established by Vsb1 are mobilized to the cytosol. We show that this export involves the vacuolar Ypq2 transporter, homologous to the human lysosomal PQLC2 protein and functioning as a facilitator. Our study shows that Vsb1 and Ypq2 play an important role in arginine import and export, respectively. It also suggests that they undergo inverse regulation according to nitrogen supply conditions.

202 Activation of TORC1 in Response to H+ Influx: Role of Pma1.

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The Target Of Rapamycin Complex 1, TORC1, is a kinase complex conserved throughout eukaryotes. It plays a central role in controlling cellular growth according to various stimuli, most importantly nutrients. TORC1's activity is typically low in nitrogen-starved cells, and it rapidly increases upon uptake of external amino acids. This is mediated via the conserved Rag family GTPases. In human cells, cytosolic amino acids control mTORC1 activity by binding to diverse proteins (Castor, Sestrin, Samtor) acting as key upstream regulators of the Rag GTPases (1). Yet these proteins, in contrast to the Rag GTPases, are not conserved in lower eukaryotes including yeast, and so how is yeast TORC1 reactivated upon amino-acid uptake into nitrogen-starved cells has long remained unclear (2). We recently reported that yeast TORC1 is controlled by a different mechanism involving Pma1, the plasma membrane H+-ATPase. Our data suggest a model whereby Pma1, once stimulated by H+ co-transported into the cells with amino acids or other nutrients, activates a signaling pathway controlling TORC1's activity (3). We will present data of ongoing experiments aimed at assessing the validity of this model.

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203 Massive parallelization as a novel approach for evolving industrially relevant microbes

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Industrially oriented evolution experiments offer GMO-free enhancements however they suffer from strain background effect, stochasticity and unwanted side effects. Most of evolution experiments are conducted with few strains in few shake flasks and therefore importance of scale is overlooked. We introduced and validated a novel evolutionary engineering platform able to follow >10000 yeast populations evolving for eight industrially relevant wine traits. Starting with 48 different wine and vineyard strains with 24 replicates of each, rather than a single starting point, provided us a vast genetic search space which resulted in endpoints that were 62% more adapted than the average. We investigated the evolution side-effect by mapping the endpoints on 18 must-based environments, variety of C and N sources, in the absence of selection pressure. Most of the selection regimes showed a parallel phenotypic evolution where a positive effect on C consumption and a negative effect on N consumption was detected as an overall pattern. In case of sugar tolerance, it seems crossing a critical variance threshold among parentals unleashed the deterministic delimitations and let a divergent phenotypic manifestation of the end-points. Validating the top end-point populations in lab-scale cultivations, we demonstrate that many show significant advantages in industrial traits, including in NCR relaxation, gluthatione production and ethanol tolerance. We produced commercial quality wine by pilot scale grape-must cultivations of nominated candidates. This proof-of-concept establishes how massive parallelization affectedly supports microbial evolutionary engineering for industrial applications.

204 Saccharomyces cerevisiae and Social Wasps as Models for the Evolution of Host Microbe Interactions.

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The recent discovery that wasps are an ecological niche of Saccharomyces cerevisiae, suggests the possibility to use insects as a model to investigate the evolution of commensalism. While previous publications showed the advantage for yeast to overwinter in insect's gut, the insect's benefit from the presence of this microorganism is currently unknown. Here we studied the interactions of previously studied gut yeast strains amongst them and with the host. Initially we performed interactions between the different strains, from different origins, within wasp's gut, measuring strain fitness and survival within the gut. We then selected two yeast strains of S. cerevisiae, previously shown to induce immune priming in mammals, and investigated their ability to enhance resistance to bacterial infection in the paper wasp Polistes dominula and shape the gut bacterial communities. The reduction of injected E. coli load by S. cerevisiae strains was statistically significant in queens but not in workers, and this occurs regardless of the season and colony cycle phase of the wasps. We then investigated the changes in gut microbiota analysis, monitoring the effects of S.cerevisiae on the wasp microbiome. We observed that administration of S. cerevisiae also influenced the composition and diversity of gut microbial communities but not the fungal communities. The two strains influenced differently the insect immunity, reflecting the differences observed in the mouse model. We thus sequenced the entire genome and the transcriptome of the two strains, highlighting the gene networks regulating pseudohyphal transition and sporulation as major drivers of the strain specific differences in immune priming. We conclude that immune priming from S.cerevisiae favors resistance to infections and fitness of foundress queens, thus suggesting the possibility of positive selection on the pathways inducing immune training. Our results support the consideration of insect as a model for studying host microbe interaction, and the evolution of the interactions between yeasts strains and the microbiome.

205 Yeast Telomeric Protein Cdc13 Binds Secondary Structures on Telomeric DNA With Low Affinity.

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Telomeres, the ends of linear eukaryotic chromosomes, often possess a 3’ single-stranded overhang. The double-stranded and single-stranded (ss) portion of telomeres are bound by different proteins; in the budding yeast Saccharomyces cerevisiae, the main player binding the ss overhang is the protein Cdc13. However, G-rich ss sequences, such as telomeric DNA, are prone to fold into Hoogsteen-pairing-based secondary structures, such as G-quadruplex or G-hairpin. In our work, we analyzed secondary structures forming on the budding yeast telomeric DNA in vitro and assessed the affinity of the DNA-binding domain of Cdc13 (Cdc13-DBD) towards these structures. Our results demonstrate that a range of secondary structures forms on the yeast telomeric overhang in vitro. These include G-hairpin and G-quadruplex structures of various topologies that exhibit diverse folding kinetics. We have shown that the affinity of Cdc13-DBD towards the secondary structures is lower compared to its ss DNA binding motifs and additionally, Cdc13-DBD does not seem to participate in their unwinding. Our results offer implications for the dynamics of Cdc13-mediated telomerase recruitment during telomere elongation.

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206 mRNA Homeostasis Perturbed: Rapid Depletion of Chromatin and RNA Processing Factors Reveals the Limits of mRNA Homeostasis.

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Cellular mRNA levels are at a dynamic equilibrium of transcription and degradation. Multiple studies in yeast show transcription and degradation rates compensate for each other to yield a similar level of cellular mRNA in various environmental and genetic conditions. However, this phenomena was mostly observed at steady-state, and the details and extent of the compensation remain poorly understood. Here, we examine the cellular response to mRNA perturbations using the auxin-inducible degradation system to rapidly remove dozens of key complexes in transcription, chromatin, and mRNA processing. Immediately following the target depletion, we use metabolic labeling with 4tU coupled to mRNA sequencing to dynamically monitor total and nascent mRNA levels genome-wide with a temporal resolution of minutes. Surprisingly, we find that cells rarely manage to re-calibrate to their initial mRNA levels, and in certain cases cells grow with as little as 20% of the WT mRNA levels. However, when Xrn1 or Dcp2 are depleted we observe a striking example of feedback in real time, wherein mRNA accumulates followed by transcription slowdown, resulting in lower cellular mRNA levels. Our results challenge the ubiquity of mRNA homeostasis, and provide detailed dynamics of the feedback and adaptation in the case of Xrn1/Dcp2 depletion.

207 The Divergent and Highly Specific Effects of the [SWI+] Prion Formation and SWI1 Deletion on the Transcriptome of the Yeast Cell

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Prions are proteinaceous infectious agents capabl of existing in at least two conformational forms of which one or more possess the ability of propagation. Being mostly associated with neurodegenerative pathological states in vertebrates, prions are usually considered as functionally inactive states of proteins mimicking loss-of-function mutations of respective genes However, universality of such inactivation among known eukaryotic prions remains elusive, if not dubious. Here we present a comparative study of the yeast Saccharomyces cerevisiae transcriptome impaired either by deletion of the SWI1 gene or prionization of the corresponding Swi1 protein, herein referred to as [SWI+]. The Swi1 protein is a part of the SWI/SNF chromatin remodelling complex, which is evolutionary conservative among eukaryotes and has an assumably non-specific mode of action; therefore, any disturbance of the Swi1 functioning is expected to lead to severe transcriptome-wide depletion. RNA-Seq analysis of the isogenic yeast [SWI+] and swi1Δ strains to the [swi-] strain showed that the SWI1 deletion leads to differential expression of 1978 genes, of which 1156 are downregulated, while presence of the [SWI+] prion upregulates 19 genes and downregulates only 140 genes. Subsequent GO and KEGG Pathway annotation shows that the SWI1 deletion impairs numerous cell activities which remain intact in both control and prionization-affected strain. For instance, protein production and degradation machinery in the swi1Δ strain suffers from downregulation at almost every stage such as RNA polymerase I and III transcription, ribosome nucleolar maturation and assembly, mRNA and unfolded protein binding, with the same being true for carbohydrate metabolism both in cytoplasm and mitochondria. What is more, deletion of SWI1 appears to upregulate gene expression in a seemingly random manner. At the same time, the effect of prion on the transcriptomic pattern does not include translation depletion and shows definite enrichment patterns in both upregulated and downregulated gene sets. However, the prion does affect carbon metabolism in the manner similar to that of SWI1 deletion. These and other obtained data are strikingly consistent with the phenotypic manifestations caused by both [SWI+] prion and SWI1 deletion, such as alleviated growth on the media containing galactose or glycerol as primary carbon source and nonsense suppression. Taken together, these data suggest that [SWI+] prion formation partially retains the Swi1 functionality as a part of the SWI/SNF complex and can be considered as both a gain-of-function and a loss-of-function trait.

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208 The Gln3 Transcriptional Regulator of the Nitrogen Catabolism Forms ‘Conditional’ Prion When Overproduced in Yeast Saccharomyces cerevisiae.

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Prions are proteins adopting two or more conformational states in the same physiological conditions, at least one of which is infectious. Typically, prion state is presented by the amyloids – protein fibrils with highly ordered spatial structure called ‘cross-beta’. In yeast Saccharomyces cerevisiae about ten prions were identified to date. Most of them belong to regulators of transcription and/or translation and contain Q (glutamine) and/or N (asparagine)-rich regions. One of the nitrogen catabolism regulators, Gln3, contains the QN-rich region that forms amyloid fibrils and being fused with reporter sequences manifests as artificial prion. Here we analyzed whether full-length Gln3 possesses prion properties. We demonstrated that overproduction of Gln3 does not cause its aggregation. Nevertheless, co-overproduction of the full-length Gln3 with its QN-rich region causes aggregation of Gln3. Moreover, Gln3 aggregation remains stably inherited in small fraction of clones after the termination of its QN-rich region overproduction. Aggregated state of Gln3 is stably inherited in mitosis, depends on antiprion agent guanidine hydrochloride and HSP104, and exhibits infectious properties being transmissible by the protein transformation. Thus, aggregated state of Gln3 satisfies criteria of the yeast prion. Unique property of the Gln3 prion state is that it propagates only when its structural protein is overproduced. Restoration of the physiological level of the GLN3 expression causes elimination of the [GLN3↑+] prion (the ‘↑’ symbol indicates that the Gln3 prion state propagates only when protein is overproduced). Phenotypically, the [GLN3↑+] prion compensates for toxicity of the Gln3 overproduction on the media containing rapamycin (the efficiency of the [GLN3↑+] strain vegetative growth is similar to the growth of isogenic yeast strain without overexpression of GLN3). We propose term ‘conditional prion’ for prions that are formed by the proteins with native amino acid sequence but propagate only under non-physiological conditions.

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209 Interactions of a Peroxiredoxin with the Proteasome in H2O2-induced Proteostasis.

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Reactive oxygen species (ROS) are assigned with disparate roles. On the one hand they can be damaging to essential macromolecules and an imbalance of ROS production and elimination has been associated with ageing processes and accompanying maladies. On the other hand ROS, especially H2O2, are employed as second messengers in various cellular processes and therefore their levels need to be regulated carefully. Mirroring the need for ROS balance, the most H2O2-reactive enzymes in vivo, peroxiredoxins, that are conserved in organisms from bacteria to humans, have been assigned multiple functions as exquisitely sensitive ROS scavengers, as signal transducers and as ROS-activated chaperones. The major cytosolic peroxiredoxin in yeast, Tsa1, is a key regulator of yeast replicative longevity (1) and while its interaction with hydrogen peroxide is well studied, less is known about the in vivo effectors executing its functions. Recent research suggested an involvement of Tsa1 in protein quality control and the ubiquitin-proteasome system (UPS) (2).

We studied the regulation of Tsa1 and the UPS upon oxidative stress by monitoring the clearance of a proteasomal model substrate in vivo, proteasomal activity in vitro and dissociation of the 20S and 19S proteasome. We find that enhanced UPS capacity was not able to rescue the UPS deficiencies of a strain deleted for TSA1. Surprisingly, despite that cells deficient in both Tsa1 and a proteasome inhibitor display boosted proteasomal activity, they still showed enhanced accumulation of a model substrate of the 26S proteasome and increased accumulation of degradation-type ubiquitination. Further findings indicating aberrant proteasomal dissociation upon oxidative stress suggest proteasomal dysregulation in cells lacking Tsa1.

Interestingly, Tsa1 also influences global levels of K63-linked polyubiquitination, which is typically not involved in proteasomal degradation, but has been implicated in the regulation of translation upon oxidative stress and in endosomal trafficking. Together these results suggest an influence of Tsa1 on processes regulated by the UPS and that the impact of Tsa1 on these processes might be related to a role in regulating protein biosynthesis.

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210 Rapid Non-targeted Metabolite Profiling of Yeast

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S. cerevisiae is a key eukaryotic model organism for systematic studies of metabolism and an important host for synthetic biology applications. As monitoring metabolite levels has a key role in testing synthetic biology constructs, there is an increasing demand to develop fast and easy-to-operate global metabolite profiling methods.

Chromatography coupled mass spectrometry (MS) methods give high metabolome coverage and sensitivity, but are time consuming and suffer from sample instability for large sample sizes. Here we describe a rapid chromatography-free workflow to provide a global overview of the ionized intracellular metabolites of yeasts.

S. cerevisiae biomass was extracted by using a polar solvent mixture and introduced into a high-resolution mass spectrometer using eluent flow. Mass spectrometer was operated in negative ionization and full-scan acquisition mode. Compounds were putatively annotated using yeast-specific metabolome databases. To confirm the intracellular origin of the annotated compounds, we used stable isotope labeled glucose feeding.

Our method enables the monitoring of more than 80 high-quality putatively annotated metabolites, representing diverse pathways, in up to 450 samples per day. Applying the method to a set of natural yeast isolates revealed substantial metabolomic diversity in wild yeasts. Finally, the approach can be used for large-scale clustering of yeast genotypes based on their metabolome profiles and screening for efficient bioproduction strains.

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211 Environmental Stresses Induce Divergent SUMOylation Responses.

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Stress is a ubiquitous part of life which disrupts cellular function and, if unresolved, can irreparably damage essential biomolecules and organelles. All organisms are subject to stress in the form of unfavorable environmental conditions including extreme temperatures, hypoxia, reactive oxygen species, or shifts in osmolarity. To survive, organisms must sense these changes then react and adapt. One highly conserved adaptive response is sumoylation, which is a post-translational modification by the small ubiquitin-like modifier (SUMO) protein. SUMO is a broadly used signaling molecule capable of altering protein localization, interactions, and solubility. In the context of variable exogenous stresses, we find that S. cerevisiae exhibit unique and dynamic patterns of sumoylation as part of a concerted effort to return the cell to homeostatic conditions. These SUMO-based stress responses vary in kinetics and targeted substrates in accordance with the type, severity, and duration of stress. We therefore conclude that, rather than employing a generalized SUMO stress response, yeast tailor their use of this highly versatile modification to suit the present environmental conditions.

212 Saturated Transposon Analysis in Yeast goes further.

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SAturated Transposon Analysis in Yeast (SATAY) is a versatile, time- and labor-efficient method to functionally explore the S. cerevisiae genome, using saturated transposon mutagenesis coupled to high-throughput sequencing [1]. SATAY allows one-step mapping of all genetic loci in which a transposon (here MiniDs [2]) can insert without disrupting essential functions. The principle relates to Tn-seq in bacteria [3] and is amenable to other yeast species [4-10].

Owing to its ease and the depth and kind of information it delivers, SATAY emerges as a method of choice to (1) reveal sets of genes necessary for growth in different genetic or environmental conditions, (2) map protein domains, and (3) uncover a variety of informative alleles, such as loss- or gain-of-functions.

We present SATAY 2.0, an improved protocol that boosts transposition 10-fold, thus massively increasing throughput. The method is now amenable to a wide variety of genetic backgrounds and can be easily performed in both solid and liquid media in multiple parallel conditions by a single experimentalist. Here I will present (1) the parallel screening for resistance and sensitivity to 20 chemicals. In addition to finding drug targets and exporters, we show that transposon insertions in promoters or terminators create loss- and gain-of-function alleles by altering gene expression. Remarkably, we also find essential genes that become dispensable under drug treatment. These unprecedented mutations add to the spectrum of alleles that are unique to SATAY; (2) the first head-on comparison of two common laboratory backgrounds, BY4741 and W303. Similarities and differences will be discussed; (3) the detection of phenotypic delays accompanying the deletion of essential genes, indicating that SATAY can be used to systematically track mutant fitness dynamics. Our preliminary data already bear interesting biological implications; (4) the identification of genes sharing common functionalities by genome-wide correlation analysis between multiple independent libraries.

Finally, I will touch upon new toolboxes that expand the method versatility.

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213 Relationship between Copy Number Change and Brewing Characteristics of Bottom Fermenting Yeast.

Tomoko Takahashi, Tomoko Koyano, Keizo Kusunoki, Taku Kato, Hideyo Tadami and Kazuhiko Uemura

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Fermentation is a significant process in beer brewing. In fermentation process, yeast converts wort to ethanol, aroma compounds and lots of other by-products. These products is a key of beer flavor, but the quantity and composition of these metabolites is influenced by the type of yeast strain and its condition. So yeast plays an important role in beer quality. In beer brewing, yeast generally is used repeatedly, but this serial repitching often causes undesirable changes of yeast characteristics such as a decrease in sugar consumption, poor flocculation and off-flavors production. Bottom fermenting yeast Saccharomyces pastorianus is seen as natural hybrids of S. cerevisiae and S. eubayanus, so S. pastorianus is aneuploidy. It is known that genome instability in bottom fermenting yeast causes genomic changes in response to environmental condition.

To reveal the relation between brewing characteristics of bottom fermenting yeasts and genomic changes, we carried out next-generation sequencing analysis of various yeast crops. Most yeast genome was stable. But several copy number changes of chromosomal regions were observed and common changes occurred in some crops. This common change were also confirmed by RT-PCR, and the copy number of specific region were reduced by 80% at the maximum as compared with fresh yeast. The yeasts with common copy number changes flocculated early during fermentation. This result indicates that copy number change of bottom fermenting yeast is contribute to brewing characteristics.

214 Multi-omics dissection of Hsp90-mediated trait plasticity

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Cancers evolve through the sequential acquisition of mutations that collectively perturb cellular homeostasis thereby unleashing havoc. How do these deranged cells survive, amass additional deleterious mutations, evade host defenses, form tumors and acquire resistance to therapies? An emerging strategy cancer cells employ to achieve these feats of evolution is the expression of phenotypic plasticity. By accommodating phenotypic plasticity, cancer cells maximize the adaptive value of existing genetic variation, reducing the need for new detrimental alterations. To this end, cancer cells receive help from a powerful ally, the heat-shock protein 90 (Hsp90). Hsp90 is a highly conserved protein-folding chaperone that assists in the folding and disposition of many proteins in the cell. In helping proteins fold and function in the cell, HSP90 allows the accumulation of detrimental genetic variation across populations and renders their manifestations conditional upon benign proteotoxic stressors in the environment. Here, we employed a multi-omics approach in the budding yeast Saccharomyces cerevisiae to study how Hsp90 renders cellular traits plastic and to understand how Hsp90-contingent traits are genetically assimilated. Integrating proteomic, transcriptomic, metabolomic, and genomic datasets with quantitative trait locus mapping reveals molecular and genetic mechanisms shared by diverse Hsp90-dependent traits. Following the assimilation of traits in diverse wild yeast species, we uncover universal principles of robustness that govern the Hsp90-dependence of cellular traits. This work reveals mechanisms of genetic accommodation and assimilation of Hsp90-dependent traits and uncovers a multi-omics strategy to improve the usefulness of Hsp90 inhibitors in the clinic.

215 eIF2 and Cdc123 in mRNA translation and cell cycle entry

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The eukaryotic translation initiation factor 2 (eIF2) plays a central role within the initiating steps of protein synthesis by delivering the initiator tRNA to the ribosome (Dever et al., 2016, Genetics 203: 65-107; Hinnebusch, 2014, Annu Rev Biochem. 83: 779-812). In addition, eIF2 plays an important role in the regulation of protein synthesis and gene expression in response to stress. Stress-induced phosphorylation of eIF2 reduces global translation but at the same time, upregulates translation of certain mRNAs like GCN4, which encodes a transcriptional activator of stress responsive genes (Hinnebusch, 2005, Annu Rev Microbiol. 59: 407-450).

Through mutational analyses in the budding yeast S. cerevisiae several CDC (cell division cycle) genes were identified whose products are required for cell cycle progression (Hartwell et al., 1970, Proc Natl Acad Sci USA 66: 352-359). Unlike the classical CDC genes, CDC123 was first described in a temperature-sensitive rat fibroblast cell line, which was not competent for a G1-S transition at the restrictive temperature (Ohno et al., 1984, Somat Cell Mol Genet 10: 17-28). Cell cycle defects were also caused by mutations of the orthologous gene in S. cerevisiae, which was therefore named CDC123 (Bieganowski et al., 2004, J Biol Chem. 279: 44656-44666). Work in our lab confirmed the requirement of Cdc123 for cell cycle entry and, moreover, showed that mutations of CDC123 reduced the assembly of the eIF2 subunits resulting in an increased GCN4 expression and at the same time to a decrease of global translation (Perzlmaier et al., 2013, J Biol Chem. 288: 21537-21546). Likewise, yeast cell cycle entry was delayed when the function of eIF2 was reduced, due to either an assembly mutation in eIF2gamma or lower levels of tRNAi. These data indicate that normal assembly and function of eIF2 is a prerequisite for unperturbed cell proliferation.

To address the underlying mechanisms, we set out to determine the translational efficiencies of mRNAs in mutants of eIF2. To this end, we compared the polysome-associated and total mRNAs for wild type and eIF2 mutant cells by RNA sequencing. To find a conserved response, we performed the sequencing analysis in the W303 and BY4741 strain background. We combine the gene expression data with genetic interaction studies and live cell imaging. A detailed view of the results will be presented on the poster.

216 Saccharomyces cerevisiae as a Model to Study Supramolecular Protein Complexes in Innate Immunity TLR Signaling.

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Toll-Like Receptor (TLR) signaling relies on the assembly of huge supramolecular complexes (SMOCs) upon pathogen-associated molecular patterns (PAMPs) recognition to trigger the innate immunity response in vertebrates. SMOCs form via interaction among particular protein domains of the components of the TLR pathway, such as Toll-interleukin-1 receptor homology domains (TIR) and Death Domains (DD), and subsequent oligomerization triggered by this initial interaction. To understand their function and role in human pathologies, it is key to unravel the hierarchy of such molecular interaction. In this work we have developed a Saccharomyces cerevisiae model for TLR-related intracellular SMOCs and were able to recapitulate in our yeast model multiple known interactions.

Several components of TLR4 signaling were expressed in yeast as either GST or fluorescent protein fusions, namely the TIR domain of the TLR4 receptor, the adaptors TIRAP, MyD88, TRAM and TRIF, the protein kinases IRAK4, IRAK1 and IRAK2 and their downstream E3 ubiquitin ligase TRAF6. By using a plasma membrane (PM)-directed version of the TLR4 TIR domain, we reconstituted TLR4(TIR)-TIRAP-Myd88 complex at the yeast plasma membrane. We also detected TLR4(TIR)-TRAM interaction, as we observed that the latter displayed PM associated curly filaments that were disrupted upon TLR4(TIR) co-expression. Moreover, mutations on key TIR domain residues resulted in loss of their ability to interact either with themselves or with other TIR domains.

The interaction of MyD88 and IRAK4 mediated by DD motifs, as well as the interaction between TRAF6 and IRAK1/2, were also reproduced on yeast. IRAK4 behaved as a constitutively active kinase when produced in yeast, inhibiting growth in a way dependent of its catalytic activity. Mass Spectrometry analyses of purified MyD88-IRAK4 complexes from yeast lysates led to the discovery of multiple phosphorylated sites in the MyD88 TIR domain, thus raising the idea of MyD88 being a substrate for IRAK4. Altogether, our results offer a new experimental setting to devise new pharmacological and genetic screening platforms to test pathologic variants of these proteins, or to perform structure-function studies on these complexes.

217 Identification and Characterization of Yeast Proteins Form Phase-Separated Condensates under Glucose Starvation Stress

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Phase separation is a process through which a well-mixed solution of macromolecules such as proteins or nucleic acids spontaneously separates into two or more phases. An accumulating body of evidence suggests that phase separation also involves in adaptive stress response. Cells can use phase separation to sense the environmental changes. Moreover, some membrane-less organelles formed by phase separation, like stress granules, provide protection for key cellular components. However, the mechanism underlying phase separation regulated stress response and components associated with the process are still not clear. Here, we use Saccharomyces cerevisiae as model to systematically identify novel proteins which formed phase-separated condensates upon glucose starvation stress and reveal their functional roles in glucose starvation stress adaption. We treated yeast cells with 2-deoxyglucose (2DG) to induce starvation stress, and then isolated proteins formed foci by a high-throughput imaging-based screening approach using the yeast GFP fused proteins collection. Identified candidates were further verified for their phase separation status. We have identified promising candidates and are now studying their phase behavior and material states with both in vitro and in vivo assays. This project will provide us a global understanding on the functional roles of phase separation in cellular stress response.

218 Hydroxycitric Acid Can Antagonize Chronological Aging, Apoptosis and ROS-Induced Cell Death in Budding Yeast

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Caloric Restriction Mimetics (CRMs) are promising molecules to prevent age-related diseases. Some bona fide CRMs have been defined as agents causing protein deacetylation and autophagy, possibly extending lifespan. Hydroxycitric acid (HCA), a nutraceutical CRM from tropical plants, is thought to mainly acts as a competitive inhibitor of ATP citrate lyase (ACLY), depleting cytosolic AcCoA and so inhibiting both protein acetylation and lipogenesis. HCA has been shown to reduce body weight, insulin resistance, inflammation and oxidative stress as well as to promote autophagy and antitumor therapy efficacy (Madeo et al. Cell Metab. 2019, 29(3):592-610).

Here we preliminarily describe some HCA effects on S. cerevisiae, where ACLY enzyme is lacking. Strikingly, the drug revealed a powerful anti-aging effect, greatly extending the chronological life span (CLS) of yeast cells in a dose-dependent way. This phenotype was related to HCA ability to repress cell apoptosis and necrosis during CLS but even after a pro-apoptotic acetic acid treatment on growing cells. The drug appeared to control the metabolism as a function of the physiological status of the cells. Indeed, HCA-treated quiescent cells resumed cell growth more slowly than controls, but then proliferated at the maximum growth rate in the presence of the drug. HCA also markedly modulated the oxygen consumption rate (OCR) of stationary phase cells before aging. Finally, it largely prevented the cellular death caused by a severe hydrogen peroxide-driven oxidative stress, as shown by FACS analysis of dihydrorhodamine (DHR) 123 /PI double stained cells.

Our system gives the unique opportunity to identify new phenotypically relevant HCA targets among metabolic enzymes and regulatory pathways. Indeed, HCA also inhibits phosphofructokinase, isocitrate dehydrogenase, aconitase, citrate synthase and pyruvate dehydrogenase whereas it can remarkably activate AcCoA carboxylase (Cheema-Dhadli et al. Eur. J. Biochem. 1973, 38: 98-102). In addition, in our preliminary analyses on CLS mutants we observed that sch9- cells were almost unresponsive to HCA benefits during aging and were able to mimic the HCA-mediated rescue from cell death caused by an oxidative stress. However, sch9- mutation did not alter the OCR changes induced by the drug. Finally, the HCA rescue ability from age-related cell death of ras2- populations was significant but partial. These results suggest that HCA can act with multiple ACLY-independent mechanisms.

219 The Paradoxical Behaviour of Xylose-utilization in Recombinant Saccharomyces cerevisiae from a Sugar Signalling Network Point-of-View.

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Sustainable fermentation of lignocellulosic biomass with S. cerevisiae is contingent on a number of challenges, including tolerance to process conditions and the inability of wild-type strains to grow on xylose (a pentose sugar consisting up to 30% of the lignocellulose feedstock). Despite many successful engineering strategies to implement and improve xylose metabolism in S. cerevisiae, the utilization rate of xylose is still substantially lower than the one of glucose. A number of studies have suggested that the recombinant strains display signs of not recognizing xylose as a fermentable sugar, based on metabolome profiles and respiratory growth patterns [1, 2]. This paradoxical behaviour on xylose – that the strains ferment xylose while the cellular signals suggest otherwise – have led us to believe that the next steps for optimization of the xylose catabolism should be directed to the sugar signalling networks.

To this end, we have constructed and applied a panel of GFP-coupled glucose signalling biosensors strains to assess how the sugar signalling networks of recombinant S. cerevisiae strains respond to natural and foreign carbon sources. In reporter strains engineered with an evolved galactose transporter with xylose affinity and the oxidoreductive xylose pathway, high concentrations of xylose (50 g/L) were found to trigger a signal similar to that of low glucose concentrations (1 g/L), which illustrates that the cell does not see xylose as a readily utilizable carbon source. Furthermore, when a number of deletions that were recently found to improve xylose utilization [3] were introduced in these strains, the signal shifted to a pattern that could be described as simultaneous sensing of low and high glucose concentrations. The present study shows that engineering of signalling is an important aspect in cell factory development that will increase the fundamental understanding of nutrient signalling of natural and non-natural carbon sources.

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220 Clotrimazole Deeply Alters MAPK Signalling in Saccharomyces cerevisiae and Other Yeasts.

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Signal transduction pathways mediated by Mitogen-Activated Protein Kinases (MAPKs) are essential for the response of eukaryotic cells to external stimuli. In the yeast Saccharomyces cerevisiae, there are five of these MAPKs routes. Among them, the Cell Wall Integrity (CWI) pathway mediated by the MAPK Slt2 responds to stimuli that affect the stability of the cell wall, a vital component of the cell, permitting the survival of the yeast.

Azole antifungals are commonly used for the treatment of fungal infections. Their mechanism of action involves the inhibition of the lanosterol 14-alpha-demethylase enzyme which catalyzes one step of the ergosterol synthesis pathway, provoking disruption of fungal membranes. They are thought to induce other detrimental effects inside cells, but as yet little has been reported about it. In this work, we demonstrate that one of these azoles - clotrimazole - activates S. cerevisiae CWI pathway leading to phosphorylation of its MAPK Slt2. We have characterized this activation and observed forms of P-Slt2 with lower electrophoretic mobility, which are directly dependent on Slt2 phosphorylation in its TEY domain and disappear after treatment with phosphatase alkaline. Consequently, SLT2 deletion renders cells sensitive to this compound, which demonstrates the important role of Slt2 in surviving after clotrimazole treatment. This sensitivity of slt2Δ strains can be partly prevented by osmotic stabilization with sorbitol, supporting that cell wall damage is underlying the detection of this stimulus. Moreover, cells exposure to clotrimazole also affects the pheromone response pathway but in a different manner, causing a strong reduction of the phosphorylated levels of its MAPKs Kss1 and Fus3.

To extend this study, we have analysed in a comparative manner the effect of clotrimazole and the different azole fluconazole on MAPK signalling of other eight additional yeast species, including some of special interest due to their implication in fungal infections like Candida albicans and Candida tropicalis.

221 The polarising world of Cdc42: the importance of geometry in cell division

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A key regulator of cell polarisation in organisms ranging from yeast to higher mammals is the Cell division control protein 42 homolog, Cdc42. It is a GTPase of the Rho family where it determines the site of the pole by a combination of reactions (i.e. activation and deactivation) and diffusion in the cell. A study in yeast showed that with high age, the Cdc42 pathway loses its function which thus prevents replicative ageing. Moreover, Cdc42 activity is involved in both ageing and rejuvenation of hematopoietic stem cells which thus illustrates the important role that Cdc42 plays in the ageing process of numerous organisms.

Experimentally, the challenge with studying the activation of Cdc42 is that the concentration profile is not uniform in the cell. In other words, most active Cdc42 aggregates at a specific location on the cell membrane due to different diffusion rates and thus accounting for spatial inhomogeneities is crucial when data of the pathway is collected. Considering that measuring two different diffusion rates simultaneously is challenging, numerous mathematical models have been developed. However, those models do not take cell geometry into account and thus do not provide a realistic mechanistic understanding of how the various constituents of the polarisation process mediated by Cdc42 interact during cell division.

In this project, we develop a quantifiable model of cell polarisation focusing on cdc42 accounting for the morphology of the cell. We show that the choice of rate parameters greatly depends on the geometry of the cell which must be accounted for when validating the model using experimental data. We will further study how the diffusion coefficients and the reaction terms impact the patters that emerge and estimate the time until polarization. Using this work as a starting point, it is possible to integrate data into the theoretical description of the process to deeper understand cell polarisation on a mechanistic level.

222 A role for the Saccharomyces cerevisiae ABCF protein New1 during translation termination

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Translation on the ribosome is controlled by numerous accessory proteins and translation factors. In the yeast Saccharomyces cerevisiae, translation elongation requires an essential elongation factor, the ABCF ATPase eEF3. A related ABCF ATPase, New1, is encoded by a non-essential gene with a cold sensitivity and ribosome assembly defect knock-out phenotype. Since the exact molecular function of New1 is unknown, it is unclear if the ribosome assembly defect is direct, i.e. New1 is a bona fide ribosome assembly factor, or indirect, for instance due to a defect in protein synthesis. To investigate this, we employed a combination of yeast genetics, cryo-electron microscopy (cryo-EM) and ribosome profiling (Ribo-Seq) to interrogate the molecular function of New1. Overexpression of New1 rescues the inviability of a yeast strain lacking the otherwise strictly essential translation factor eEF3. The structure of the ATPase-deficient (EQ2) New1 mutant locked on the 80S ribosome reveals that New1 binds analogously to the ribosome as eEF3. Finally, Ribo-Seq analysis revealed that loss of New1 leads to ribosome queuing at C-terminal lysine and arginine residues, including genes encoding proteins of the cytoplasmic translational machinery. Taken together, our results suggest that New1 is a translation factor that fine-tunes the efficiency of translation termination.

223 Acquisition of Genotoxin Resistance through Checkpoint Adaptation and Aneuploidy

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In response to DNA damage cells activate checkpoints in order to arrest cell cycle progression and repair their DNA. When the DNA damage cannot be repaired, cells eventually divide in the presence of DNA damage, a phenomena known as checkpoint adaptation. Although initially described in budding yeast Saccharomyces cerevisiae, adaptation has been observed in higher eukaryotes, including human cells. The risks associated with checkpoint adaptation include massive genomic instability and cell death. A critical driver of checkpoint adaptation is the polo-like kinase Cdc5PLK1.

Mutations in genes involved in homology-directed repair pathways render cells unable to repair double strand breaks, and cells undergo checkpoint adaptation after chronic DNA damage in such a genetic context. We study checkpoint adaptation in diploid S. cerevisiae that lack the Rad52 recombinase. The repair defect in rad52 yeast highly resembles that of BRCA1-/- and BRCA2-/- human cancers that are known to be recombination defective with high levels of genomic instability, and frequently develop resistance to genotoxins. We are able to model this scenario in yeast, as repair-defective rad52 cells, when challenged with genotoxins, exhibit extensive chromosome loss and eventually acquire resistance to further treatments. However, the role checkpoint adaptation plays in the survival of repair-defective cells upon genotoxic stress conditions and in acquisition of resistance to genotoxins is unclear.

Checkpoint adaptation can be prevented genetically using an adaptation-defective cdc5-ad allele or pharmacologically by using the TORC1 inhibitor, rapamycin. We show that the prevention of checkpoint adaptation sensitizes repair-defective rad52 cells to treatment with campthotecin and X-Ray irradiation, thereby decreasing the occurrence of genotoxin-resistant colonies. We further find that MRXMRN complex plays important role in survival of rad52 cells on Campthotecin. Moreover, we demonstrate that adapted cells suffer from aneuploidy-associated phenotypes, which can be further targeted pharmacologically. Our study provides a critical link between checkpoint adaptation and acquisition of genotoxin resistance by repair-defective cells.

224 Development of a Biosensor for the Plasma Membrane Lipid Asymmetry.

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In the plasma membrane lipid bilayer, lipid composition differs between the inner and the outer leaflet. This phenomenon is called lipid asymmetry and conserved in eukaryotes. Maintenance and proper regulation of lipid asymmetry is essential for cell viability because it plays a central role in a great variety of cellular events, including generation of membrane potential, vesicular transport, polarized cell growth, and cell migration. Despite such importance, no biosensors that can report the state of lipid asymmetry in living cells is available at present, being a bottleneck in the research. Using the yeast S. cerevisiae, we previously discovered a sensor protein, Rim21, that monitors the state of lipid asymmetry. We then identified the sensor motif, within the cytosolic region of Rim21, for lipid asymmetry. Based on these findings, we tried to develop a biosensor for lipid asymmetry. We will present the results of this project in this conference.

At first, we constructed a prototype of the biosensor by fusing GFP with the sensor motif moiety. Using this prototype, we succeeded in visualizing alterations in lipid asymmetry in living yeast cells for the first time. The biosensor was attached to the inner leaflet of the plasma membrane in WT cells, but it dissociated from the plasma membrane in mutant cells with disturbed lipid asymmetry. Super-resolution microscopy of the living yeast cells revealed that the state of lipid asymmetry is not uniform but locally differs within the plasma membrane. To develop a series of lipid asymmetry biosensors with a different sensitivity and dynamic range, we performed a mutational analysis of the prototype. By introducing mutations in the conserved motif near the lipid asymmetry sensor motif, we succeeded in constructing improved biosensors that report the state of lipid asymmetry more clearly and with higher contrast. Using the prototype and the improved versions of the biosensor, we monitored the state of plasma membrane lipid asymmetry in living yeast cells exposed to environmental stresses. We observed dissociation of the biosensors from the plasma membrane in response to alkaline and salt stresses, suggesting that these stresses cause disturbances in lipid asymmetry. Interestingly, the lipid asymmetry sensor protein Rim21 is known to be involved in adaptation to alkaline and salt stresses. We propose that these stresses are sensed by Rim21 at least partly through alterations in lipid asymmetry.

225 Development of two Acetic or Lactic Acid Biosensors in Yeast.

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Acetic acid, formed during the hydrolysis of biomass, is highly toxic and inhibitory for yeast already at concentrations of 5-10 g/L. Acetic acid is able to diffuse over the cell membrane causing multiple damages and homeostasis perturbation, slowing down metabolism and eventually leading to cell death and lower productivity as the number of active cells decreases. In lignocellulose hydrolysates, the acetic acid concentrations may be >10 g/L, making it a major challenge in developing an efficient yeast host for conversion of lignocellulosic hydrolysates.

Lactic acid is commonly used in several industries and its market demand is higher than the effective production. In industrial production, low pH is preferred to produce the lactic acid in its protonated form and at high concentrations. Nevertheless, the problem of cell toxicity is still present at low pH or at high concentrations of lactic acid, representing a major cause of stress for yeast. For this, the development of biosensors capable of detecting the concentration of acetic or lactic acid will represent a useful tool for the detection of strains with higher production and/or tolerance towards these two compounds.

A biosensor can be defined as a molecular device used for the detection of a specific compound inside or outside a cell. The biosensor produces a quantifiable signal in response to its target molecule. The biosensors to be developed in this work will be based on a transcription factor, activated by either acetic or lactic acid and capable to generate a fluorescent signal, proportionally to the intracellular concentration of the targeted compound. These molecular devices will be initially integrated into a laboratory strain, using techniques of both rational and evolutionary engineering to improve their performances. The optimization of the biosensors will subsequently allow their employment for comparisons between different industrial strains, eventually leading to the identification of more efficient hosts for bio-industrial production.

226 Metabolic Buffering against Proteotoxic stress in Aging Cells.

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In this study, we investigate the influence of intracellular metabolite composition and levels, on the cellular protein quality control of young and replicative old cells of Saccharomyces cerevisiae. Since, cellular metabolites can act as chemical chaperones, we hypothesized that, an inability to accumulate beneficial metabolites with age or accumulation of harmful metabolites may result in altered proteostasis capacity. In a preliminary study, we identified metabolites that show increased accumulation in replicative old cells of wild type strain BY4741, compared with young cells and chose L-serine as the target metabolite. Since, L-serine has previously been shown to accelerate refolding of two model proteins in vitro, its accumulation in old cells could have important implications on cellular protein quality control. In order to test this, we chose yeast deletion strain CHA4Δ. CHA4 is a transcriptional activator that regulates intracellular L-serine by repressing its biosynthesis by SER3 (3-phosphoglycerate dehydrogenase and alpha-ketoglutarate reductase enzyme) and activating its catabolism by the CHA1 (catabolic L-serine (L-threonine) deaminase enzyme. We observed that the CHA4 deletion leads to accumulation of intracellular L-serine even in young cells. Using two unrelated misfolding prone proteins (Guk1-7ts-GFP and luciferase-GFP) and observing their aggregation behaviour, we show that even the young cells of CHA4Δ strain has compromised protein quality control compared with the wild type. Further, we confirmed that the effect of CHA4 deletion is general and not restricted to the model proteins used, by observing the aggregation of endogenous proteins using Hsp104-GFP. CHA4Δ strain was observed to have increased percentage of old cells with aggregates of endogenous proteins, compared with the wild type. In conclusion, L-serine accumulates in old cells and perturbing L-serine metabolism leads to altered cellular proteostasis capacity.

227 Structural Changes in Heat-stressed Yeast Cells.

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Yeast cells subjected to heat stress is often used as a model to study cellular processes occurring during cellular aging. The field has now gained a lot of insight into the genetics, molecular and cellular biology of this cellular stress response. Therefore, it is now essential to study the structural changes that occur when a yeast cell is subjected to heat stress.

To image organelle integrity in a near native manner we used high pressure freezing followed by freeze substitution (HPF-FS) of Saccharomyces cerevisiae which was then visualized using transmission electron microscopy. 3D immuno-electron microscopy was performed to localize Hsp104, a protein known to bind and disaggregate protein aggregates accumulating during stress.

Our structural investigation shows dramatic differences in a variety of cellular structures. The most prominent of those is a shift from electron dense to electron translucent vacuoles with various electron dense precipitates inside. The change from electron dense to electron translucent vacuole correlates with an increase in vacuolar pH visualized using pH sensitive fluorescent vacuolar probes. This indicates that our HPF-FS protocol might serve as an indicator of intraorganellar pH. There was also an accumulation of membrane-less electron translucent clusters in cells subjected to heat shock, these clusters are sometimes seen in publications of stressed cells even though they are rarely discussed. Our immune-labelled 3D reconstructions of heat shocked cells showed clusters of Hsp104 localizing near mitochondrial surfaces, displaying local hubs of protein aggregation in the cytoplasm.

Using cryo-preparation methods of cells too large for cryo-electron microscopy have yielded important cell biological insights into the yeast stress response to heat. This will further lead to a better understanding of the mechanisms behind various cellular processes also occurring during cellular aging.

228 Role of the Peroxiredoxin Tsa1 in Protein Biosynthesis

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We and others found that peroxiredoxins, conserved tumor suppressors, hydrogen peroxide scavengers and signaling enzymes, counteract aging and age-related decline in several organisms including yeast upon stimulation by caloric restriction (reduced caloric intake without malnutrition) or CR mimetic drugs. All eukaryotic organisms have evolved to use oxygen-dependent metabolism, as excess of its oxidizing powers produces toxic compounds, called reactive oxygen species (ROS). In S. cerevisiae there is a large group of ROS detoxifying enzymes, including the peroxiredoxin Tsa1. However, we found that Tsa1 appears to control aging independent on the levels of H_2O_2. In fact, the addition of low levels of H_2O_2 slows down aging in a Tsa1-dependent manner. Supporting this, more and more examples of ROS in endogenous signaling are described, in yeast as well as mammals. Furthermore, we have previously described a key role of Tsa1 in age-related proteostasis.

Our ongoing studies indicate that the peroxiredoxin Tsa1 has a role in regulating protein biosynthesis and that this role may regulate aging. We used mutants deficient in TSA1 and overexpressing TSA1 to study the role of Tsa1 in translation. Using puromycin, an aminonucleoside antibiotic that incorporates at the C-terminus in nascent polypeptides, we found that upon oxidative stress with H_2O_2 the tsa1Δ mutant keeps incorporating puromycin suggesting defective regulation of protein biosynthesis. We also used a next generation sequencing approach to non-invasively measure protein translation, 5PSeq, and found differences in global ribosome occupancy in strains lacking and overexpressing TSA1. In addition, we were able to measure ribosome pausing at specific codons and found differential Tsa1-dependent pausing at tryptophan codons. We have seen that excess external tryptophan was able to suppress the sensitivity of tsa1Δ mutant strains to H_2O_2 stress. However, we found no difference in tryptophan levels in the tsa1Δ mutant in the absence of H_2O_2. On the contrary, we did, however, find reduced tryptophan tRNA levels in the tsa1Δ mutant as well as defective repression upon H_2O_2 addition and are currently looking into to what extent modulating tRNA tryptophan levels affects Tsa1-dependent phenotypes.

229 Identification and Function of Novel Peroxisome Contact Sites in the Yeast Hansenula polymorpha.

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Contact sites are regions where two membranes closely associate (distance less than 30 nm). We study the occurrence and role of peroxisomal contact sites in the methylotrophic yeast Hansenula polymorpha. In this organism peroxisomes are required for growth on methanol as sole carbon and energy source. Peroxisomes are single membrane organelles that occur in almost all eukaryotic cells. Common peroxisome functions include lipid and hydrogen peroxide metabolism.

Recent electron microscopy studies showed that in H. polymorpha at peroxisome repressing growth conditions (glucose) peroxisomes form contacts with the endoplasmic reticulum and plasma-membrane. Upon shifting the cells to media containing methanol, membrane contacts are formed with mitochondria and the vacuole as well (Wu et al., 2019).

We characterized the contacts that occur between peroxisomes and the vacuole, endoplasmic reticulum and the plasma-membrane. These studies indicated that the association of the organelle with the plasmamembrane is important for peroxisome retention in the mother cell, whereas the contacts with the endoplasmic reticulum and vacuole likely are important for non-vesicular lipid transport to the peroxisomal membrane.

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230 Construction of a Xylan-degrading Industrial Strain of Saccharomyces cerevisiae with Optimized Expression and Secretion of Xylanolytic Enzymes for Bioethanol Production.

Iván Francisco Ciklic, Venkat Rao Konasani and Cecilia Geijer

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A transition towards a biobased economy is needed to lower our dependence on fossil resources. In such a future bioeconomy, plant biomass, or lignocellulose, will be an important starting material for production of fuels, chemicals and materials. The energy in lignocellulose is stored in three different carbon polymers; cellulose, hemicellulose and lignin. Hemicellulose is mainly composed of xylan, which can comprise up to 35% of the total dry weight of plants. During bioethanol production, xylan is converted to xylose primarily through the biomass pretreatment step. Unfortunately, during this process also various inhibitory compounds are formed that are detrimental for the subsequent steps of enzymatic hydrolysis and yeast fermentation. Mild pretreatment conditions result in a reduced inhibitory burden but also in an incomplete breakdown of the xylan polymers. The aim of this study is to construct an industrial strain of S. cerevisiae that can efficiently degrade and ferment xylan. This strain can be used to ferment mildly pretreated biomass, which will have the potential to improve process yields, productivity, cost effectiveness and hence the market competitiveness of lignocellulosic bioethanol. To optimize the secretion of endoxylanase XYN2 from T. reesei and β-xylosidase Xyla from A. oryzae, we are currently evaluating two alternative signal peptides, α-mating factor PCLsp [1] (a mutant of wt α-mating factorsp) and SED1sp [2], plus wt α-mating factorsp as a control. All different signal peptide/gene combinations are being evaluated with enzyme activity assays. Multiple gene copies of the best performing combination will be then integrated into the yeast genome using a CRISPR/Cas9-based method, which also allows for fine-tuning of the gene copy number to achieve ideal expression levels of the corresponding enzymes.

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231 Systematic Identification of Genes whose Overexpression Positively Affect Fitness in Various Environments.

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In order to operate biological functions most efficiently in various environments, diverse parameters in cell systems are optimized, and the gene expression levels that are determined by the synthesis of mRNA and proteins via transcription and translation are strictly regulated. Overexpression of genes generally causes negative effects by disturbing the harmony of cell systems. On the other hand, it could also affect cell functions in a positive manner in specific conditions. However, there are few systematic investigations to identify genes whose overexpression positively affect cell fitness in various environments.

Here we developed an experimental scheme that systematically surveys budding yeast genes whose overexpression positively affects cell fitness in various environments. For the purpose, we used the gTOW6000 collection in which each of all S. cerevisiae genes is cloned on a 2 μ plasmid pTOWug2-836 and maintained in a wild-type strain BY4741 (Makanae et al., 2013). The collection strains were mixed and competitively cultured in various environments. The plasmids were isolated from the cells and frequencies of the insert genes were determined by the Nanopore sequencer. Genes governing the majority of reads can be considered as the genes whose overexpression positively affect cell fitness in the given environments.

As a test case, we first performed this experiment with 1 mM methotrexate (MTX) and confirmed that DFR1 and FOL2, the targets of MTX, gave the majorities of the plasmid reads in the duplicated experiments. We also performed this experiment in other conditions such as synthetic medium, low-glucose, stationary phase, and high-temperature conditions. We would like to discuss how identified genes in these conditions function in positive ways.

232 The SAGA/TREX-2 Subunit Sus1 Plays a Significant Role in the Adaptive Transcriptional Response Mediated Through the CWI Pathway in Saccharomyces Cerevisiae.

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The Sus1 protein in Saccharomyces cerevisiae has a broad role in mRNA biogenesis as it couples and coordinates transcription and mRNA export processes, making gene expression more efficient. This evolutionarily conserved protein is both a component of the transcriptional co-activator SAGA (histone H2B deubiquitylation module: Sus1, Sgf11, Sgf73, Ubp8) and the nuclear pore-associated TREX-2 complex (Sus1, Sac3, Sem1, Thp1).

The transcriptional program triggered by cell wall stress in S. cerevisiae is mainly mediated by the cell wall integrity (CWI) pathway through the Mitogen-activated protein kinase (MAPK) Slt2 and the transcription factor Rlm1. The main objective of this work is to characterize the functional role of the protein Sus1 in the transcriptional response under stressful situations affecting the integrity of the S. cerevisiae cell wall structure.

Microarray analyses revealed that 93 genes were induced upon treatment with Congo Red (CR) in a WT strain, being almost 70% Sus1-dependent genes. RT-qPCR experiments confirmed that the expression levels of several dependent CWI genes were significantly reduced respect to a WT strain in a sus1Δ mutant under stress conditions. In addition, the impact of the different subunits of the SAGA and TREX-2 complexes on the transcriptional response in stress situations was evaluated by β-galactosidase assays using the transcriptional reporter MLP1-lacZ, as well as the effect of these mutations on the levels of the Mlp1 protein by flow cytometry using the MLP1PRO-MLP1-GFP fusion. A very significant reduction in the expression of MLP1 was observed in the presence of CR in the mutants analysed, most notably in sus1Δ, sac3Δ, thp1Δ and sgf73Δ. A defect in signalling mediated by the CWI pathway was ruled out since the activation of Slt2 was not altered in the analysed mutants grown in the presence of CR. Moreover, through ChIP experiments, it has been possible to determine in vivo the recruitment of Sus1 to the promoter and coding regions of MLP1 under stress conditions in a wild type, which is both dependent on the SAGA (Ubp8) and TREX-2 (Sac3) complexes, as well as on CWI pathway's elements (Slt2, Rlm1). From a functional point of view, the sus1Δ mutant is sensitive to different types of damage on the cell wall. All this data suggests that the TREX-2 and SAGA complexes play an important role in the adaptive transcriptional response mediated through the CWI pathway.

233 Production of Medium-Chain Fatty Acids and Derivatives in Yeast Cell factories.

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Medium-chain fatty acids (6-12 carbons) are important industrial chemicals used to produce a range of commercial molecules, which serve as oleochemicals for coatings, plasticizers, soaps, detergents, lubricants and flavors, or transportation fuels alternative to current gasoline and jet fuels. Rewiring microbial cell metabolism via using metabolic engineering and synthetic biology tools would provide a sustainable way to economically and steadily produce these kinds of molecules from lignocellulosic biomass based raw materials. And the yeast Saccharomyces cerevisiae is selected as the microbial cell factory for these products based on some superior features, such as easily accessible genetic manipulation tools, well-characterized cellular genetic, metabolic and regulatory processes, simple culture media, and inexpensive industrialization via using the existing infrastructures of bioethanol. In this presentation, I will first introduce a novel protein engineering approach used to modify the fungal type I fatty acid synthases for fatty acid chain length control, and then some strategies for the improvement of cellular fitness against toxic medium-chain fatty acids, as well as optimization of the downstream fatty acid processing pathways for the production of medium-chain alkanes, 1-alkanes, fatty alcohols and esters.

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234 The ER Membrane Chaperone Shr3 Co-translationally Assists Biogenesis of Related Polytopic Membrane Protein Substrates.

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Polytopic membrane proteins with multiple transmembrane segments (TMS) co-translationally insert into the endoplasmic reticulum (ER) membrane of eukaryotic cells. Discrete sets of polytopic membrane proteins in Saccharomyces cerevisiae require ER membrane-localized chaperones (MLC) to prevent aggregation and fold properly. Shr3, the best characterized MLC, is specifically required for the functional expression of amino acid permeases (AAP), a family of transport proteins comprised of twelve TMS. We performed comprehensive scanning mutagenesis and deletion analysis of Shr3 combined with split-ubiquitin approaches to probe chaperone-substrate (Shr3-AAP) interactions in vivo. We report a surprisingly low level of sequence specificity underlies Shr3-AAP interactions, which initiate after the first 2 to 4 TMS of AAP partition into the membrane. The Shr3-AAP interactions successively strengthen and then weaken as all 12 TMS are inserted. Thus, Shr3 acts transiently in a co-translationally manner to prevent TMS of translation intermediates from engaging in non-productive interactions, thereby preventing AAP misfolding during biogenesis.

235 Expression of the ABC Transporter Pdr18 also Increases Yeast Thermo- and Osmo- Tolerance: Underlying Mechanisms and Implications for VHG Fermentations

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The development of superior industrial yeast strains more tolerant to multiple chemical and physical relevant stresses is essential for the development of sustainable bioprocesses. During very high gravity (VHF) fermentations conducted at high process temperatures, yeast performance and final ethanol production depends on yeast ethanol tolerance, thermotolerance, osmotolerance and tolerance to several other growth inhibitors produced during fermentation or present in biomass hydrolysates.

Among the mechanisms by which yeast overcomes multiple stresses is the expression of plasma membrane transporters that confer resistance to multiple drugs/xenobiotic compounds (MDR/MXR) presumably by mediating their active efflux from the cell. However, the physiological role of these putative efflux pumps in yeast cell adaptation and tolerance to multiple stresses is still a matter of debate [1,2]. Some ABC transporters were found to mediate the reduction of the intracellular accumulation of toxicants while playing crucial roles in yeast cell physiology, such as in lipid transport [1]. This is the case of Pdr18, that confers tolerance to a wide range of chemical compounds of biotechnological interest, such as alcohols, organic acids, heavy metals and agricultural pesticides [1,3–5]. Pdr18 was found to play a role in ergosterol transport at the PM level, contributing to the maintenance of its organization and reduced permeability under acetic acid stress [5].

This work reports a new role for Pdr18 in increasing yeast tolerance to osmotic and thermal stress and their combined deleterious effects. The expression of PDR18 was found to be advantageous to attain higher final titers of ethanol under challenging conditions (300 g/L, 30ºC and 40ºC). However, yeast cells devoid of Pdr18 exhibit a higher ethanol production rate and yield under unstressing growth conditions. The underlying mechanisms were further investigated, focusing on: i) the role that a lower-ergosterol-content PM composition may have on glucose transport across membrane embedded Hxts transporters; ii) ATP savings due to the lack of Pdr18 transporter activity and/or to a less-energy demanding PM lipid composition and iii) other alterations of yeast metabolism.

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236 Genome characterization of the xylose-fermenting yeast Candida intermedia

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The urgency to reduce carbon emissions and to lower our dependence on oil makes it necessary to strive towards a more sustainable bio-based economy, where energy, chemicals, materials and food are produced from renewable resources such as lignocellulosic biomass. D-xylose, the second to glucose most prevalent sugar in lignocellulose, is an underutilized resource due to the inefficient fermentation of this sugar by the most industrially relevant microorganisms (e.g. Saccharomyces cerevisiae). Native xylose-utilizing yeasts represent a major source of knowledge and genes for xylose uptake and assimilation that can be transferred to other microorganisms including S. cerevisiae. The yeast Candida intermedia is an interesting candidate to characterize further, as it displays a high xylose transport capacity and multiple xylose reductases, of which one appears to prefer NADH over NAPDH.

The aim of this study was to elucidate the genetic features that are the basis of the xylose utilization capacity of C. intermedia CBS141442. PacBio sequencing and de novo assembly of the genome revealed a haploid yeast with a genome size of 13.2 Mb and a total of 5936 protein-coding genes spread over seven chromosomes. In order to gain insight into which genes are involved in the utilization of xylose, we compared the transcriptomic profiles of C. intermedia CBS141442 during growth in 20 and 200 g -1 of xylose and glucose. We identified 7 distinct clusters of co-regulated genes, a number of new genes potentially encoding xylose transporters and no less than three xylose reductases genes with different expression patterns. The xylose reductase genes were heterologously expressed in S. cerevisiae to determine their co-factor preferences and substrate specificities. Whereas two of the enzymes are strictly NADPH-dependent, the third can use both co-factors and shows preference for NADH. The heterologous expression of this gene can improve the capacity of S. cerevisiae to ferment xylose, and thus contribute to a more efficient use of lignocellulosic biomass.

237 The Saccharomyces cerevisiae Pan-genome

Gang Li, Boyang Ji and Jens Nielsen

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The genotype-phenotype relationship is a fundamental question in biology. Pan-genome, which accounts for a set of genes in a given list of genomes, precisely captures the protein-coding variation across these genomes and can be further used to assess the effect of protein-coding variation on phenotypes. With the benefit from the next-generation sequencing and high-throughput phenotyping methodologies, there have been a substantial genome and phenome data for Saccharomyces cerevisiae. This makes it an excellent model system to understand the genotype-phenotype relationship. Here, we performed a holistic reconstruction of the pan-genome of Saccharomyces cerevisiae from 1,392 genomes. We observed that around 20 genomes in the published datasets were contaminated by prokaryotic genomes. After removal of contaminated genes and genomes, the final pan-genome is composed of 7,078 genes. There are three distinct gene groups: (1) 74.8% genes are present in at least 95% of all genomes (extended core genome); (2) 15.8% genes are present in 5% or less (accessory pool) and (3) the rest (character genes). We found that 767 industrial strains belonging to six different types can be accurately classified with only gene presence/absence information by machine learning with an accuracy up to 90%. This indicate that in addition to previously reported sequence variations in the core genome, gain/loss of specific genes also plays an important role in the differentiation of different strain types. In conclusion, this study firstly reconstructed the S. cerevisiae pan-genome and then demonstrated its application in resolving genotype-phenotype relationships with machine learning.

238 Identifying Genes Causing Genome Instability upon Overexpression in Saccharomyces cerevisiae.

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Accurate replication of the genome is essential for cell survival. The combination of both exogenous and endogenous sources of replication stress and DNA damage serve as a constant threat to the fidelity of this process. Fortunately, eukaryotic cells have a highly conserved DNA damage and replication checkpoint, which acts to recognize and repair damage to prevent genome instability. Though the mechanisms of genome maintenance have been extensively studied in Saccharomyces cerevisiae, almost all previous screens used to identify novel genes involved in these processes have employed loss-of-function alleles, subsequently ignoring the consequences of gene overexpression. These consequences are of particular interest as several disease states are associated with gene overexpression or gain-of-function mutations, including many human cancers. Here, we assay expression of the DNA damage-inducible gene, RNR3, using reporter synthetic genetic array methodology to identify genes causing genome instability when overexpressed. We find 177 of ~5100 genes screened result in increased RNR3 expression. These genes are enriched for biological processes related to chromosome segregation and cell cycle. Around 60% of the hits in our screen are not found in published genome instability screens demonstrating that our screening strategy has the potential to identify new genes involved in genome maintenance biology. Importantly, 117 of the 177 identified hits have known human homologs and among those, 28 have been shown to be functional in yeast-human complementation studies and 4 have been identified as critical genes involved in cancer. Follow-up work will allow us to understand better the function of these new genes in the maintenance of genome instability and in carcinogenesis.

239 Intragenic Repeat Expansions Control Yeast Chronological Aging.

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Aging varies among individuals due to both genetics and environment but the underlying molecular mechanisms mostly remain unknown. Using a highly recombined Saccharomyces cerevisiae population, we found 30 distinct Quantitative Trait Loci (QTLs) that control chronological life span (CLS) in calorie rich and calorie restricted environments, and under rapamycin exposure. Calorie restriction and rapamycin extended life span in virtually all genotypes, but through different QTLs. We tracked the two major QTLs to massive expansions of intragenic tandem repeats in the cell wall glycoproteins FLO11 and HPF1 which caused a dramatic life span shortening. Life span impairment by HPF1 expansion was partially buffered by rapamycin but not by calorie restriction. We found HPF1 repeat expansion to shift yeast cells from a sedentary to a buoyant state, thereby increasing their exposure to surrounding oxygen. The higher oxygen exposure perturbed methionine, lipid, and purine metabolism, which likely explains the life span shortening. We conclude that fast evolving intragenic repeat expansions can fundamentally change the niche architecture with profound effects on cellular life span.

240 Characterization of theactivity regulation of Mep-Amt-Rh ammonium transport proteins by TORC1-Npr1-Amu1/Par32 in Saccharomyces cerevisiae

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Ammonium transport across cell membranes is ensured by Mep-Amt-Rh superfamily proteins, conserved from bacteria to human, and represented by three members(Mep1-3) in Saccharomyces cerevisiae.The inherent activity of all three Mep is controlled by the conserved TORC1 signaling pathway and its effector kinase Npr1. 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.The activity of Mep1 and Mep3 is in contrast controlled by a specific inhibitory partner, Amu1/Par32 regulated by TORC1-Npr1. Under poor nitrogen supply, Npr1 promotes phosphorylation of Amu1 which appears mainly cytosolic while Mep1 and Mep3 are active. Upon preferred nitrogen source supplementation, TORC1 upregulation enables Npr1 inhibition and dephosphorylation of Amu1 which accumulates at the cell surface and mediates the inhibition of Mep1 and Mep3.To characterize in molecular details the Mep1/3 regulation mediated by TORC1-Npr-Amu1, we designed a genetic screen to isolate suppressors recovering Mep1 ammonium transport activity in the absence of Npr1. Hundreds of suppressors were characterized. We will present the structure-function analysis of 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.

241 Precise Genome editing in Methylotrophic Yeast by Enhancing Homologous Recombination

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Methylotrophic yeasts such as Pichia pastoris and Hansenula polymorpha have been wildly used as microbial cell factories for biomanufacturing, which provides a great opportunity to establish a methanol biotransformation process to relieve the food security stresses in current sugar based bio-refineries1. In spite of so many excellent properties, it is still challenging in engineering these non-conventional yeasts due to serious lack of genetic editing tools in compared with the modeling yeast S. cerevisiae with numerous advanced genetic tools and biological devices. A crucial step in genome editing is the introduction of double-stranded breaks (DSBs) at the target loci. Afterwards, the DSBs can be repaired in two major patterns: non-homologous end joining (NHEJ) or homologous recombination (HR). In compared to S. cerevisiae with high HR efficiency, NHEJ is the dominant repairing mechanism in methylotrophic yeasts, which seriously hampers the precise rewiring the metabolic pathways in these non-conventional yeasts2.

In this presentation, we will show our recent progress in establishing CRISPR-Cas9 based genome editing tools in methylotrophic yeasts. We enhanced HR efficiency by overexpressing recombination relating genes, and also repressed NHEJ by dynamically repressed the relating enzyme Ku80 or Ku70. These engineering endeavor improved the HR efficiency 2-4 fold and enabled genome integration of multi DNA fragments. With this genome editing platform, we engineered Pichia pastoris and Hansenula polymorpha for overproduction of fatty acids at 0.5-1.5 g/L in shake flasks.

242 The Hsp70 Homolog Ssb: a Novel Component of the TORC1/Sch9 Signaling Network

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The Hsp70 chaperone Ssb and its cochaperone RAC serve a dual role in protein synthesis. First, Ssb/RAC bind to the ribosome directly and together facilitate the folding of newly synthesized polypeptide chains (1,2). Second, Ssb/RAC are required for ribosome biogenesis, such that in the absence of the chaperone system yeast cells contain a reduced number of ribosomes, display enhanced aggregation of ribosomal particles, and display subtle structural changes within core ribosomal particles, which manifest in reduced translational fidelity (2,3).

We found that the role of Ssb/RAC in ribosome biogenesis is causally linked to a moonlighting function of Ssb/RAC in the regulation of the TORC1/Sch9 signaling pathway (4). The AGC family kinase Sch9 is required to warrant sufficing ribosome production in fast growing yeast cells (5,6). Ssb is a posttranslational component of the TORC1/Sch9 signaling pathway and is required for the proper TORC1-dependent phosphorylation of Sch9 at T737, which activates Sch9, and turns on ribosome biogenesis. Our data reveal that ribosome aggregation is not confined to cells lacking Ssb but also occurs in cells lacking Sch9, indicating that Sch9 activity is required to prevent ribosome aggregation. Moreover, a permanently active Sch9 mutant partially suppresses ribosome aggregation in cells lacking Ssb (4). The combined findings reveal that Ssb/RAC does not only act via its general foldase activity towards newly synthesized polypeptides, but also posttranslationally to ensure proper signaling via the TORC1-Sch9 pathway.

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243 The Anti-Cancer Drug Zeocin Affects Copper/Iron-Regulated Transcription and Causes Metabolic Reprogramming in S. cerevisiae.

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Our research group is interested in the mechanisms of gene regulation, in response to environmental changes, through specific transcription factors and chromatin regulators. In particular, we focus on the regulation of Metal-binding Activator (Mac1), whose functionality is affected primarily by the availability of copper ions in the cell.

We have, recently, identified a specific link between, the widely used anticancer, radiomimetic and antibiotic drug Zeocin and the copper/iron homeostasis in yeast. Our findings suggest that, Zeocin causes functional deregulation of Mac1 transcription factor, apart from inducing DNA damage. Specifically, we have evidence for a functional interference of the drug with Mac1's DNA binding ability to target promoters .

Exploring the transcriptional profile of S. cerevisiae, we found that the total number of Mac1-regulated genes were down-regulated, exclusively in the presence of Zeocin, compared to other DNA damaging sources. We also found a Zeocin-specific negative effect on the conserved signaling pathway TORC1, revealed by the downregulation of ribosome biogenesis genes, upregulation of genes vital for mitochondrial functions, as well as, autophagy-related genes. Overall, the presence of Zeocin appeared to result in a switch of metabolism towards catabolism. In agreement with these findings, we have demonstrated experimentally that the drug affects a specific, TORCI-associated, kinase that phosphorylates Mac1 transcriptional activator at its DNA binding domain, important for its function.

Our results so far, establish a functional link between the copper-dependent Mac1-regulated transcription and the TORC1 signaling pathway. Moreover, they indicate two new effects of the drug Zeocin apart from its role in DNA damage induction. First, Zeocin disturbs copper/iron-regulated homeostasis by inhibiting the DNA binding function, of Mac1 transcription factor. Second, Zeocin possibly induces metabolic reprogramming in the S. cerevisiae cell, through the TORC1 protein complex function, an observation with potential promising biomedical applications.

244 An SGA-Based High-Content Microscopy Screen To Identify Genes That Control Protein Aggregate Handling During Aging.

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Aging is a ubiquitous process in life. It affects most living organisms, in particular animals but also many unicellular organisms. Aging is characterized by a time-dependent functional decline leading ultimately to death of the organism. The molecular causes of aging are still weakly understood. One of the organisms that contributed the most to aging research is the budding yeast Saccharomyces cerevisiae, which was used in this study. Despite its unicellular nature and simplicity, it exhibits an aging phenotype, which shares many properties with more complex organisms. During aging of budding yeast, protein aggregates accumulate with time, which is considered a hallmark of aging, giving rise to a collapse of essential cellular functions. This can be attributed to a combined effect of a decreased capacity of the protein quality control system (PQC) to remove aggregated proteins together with elevated levels of damaging agents during aging. As an additional protection mechanism to the PQC, the coalescence and sequestration of aggregates into large inclusions at certain protective cellular positions has evolved. However, the ability of yeast cells for aggregate coalescence and to form inclusions declines with aging, as well. Why this is the case is still unknown and could be the results of a simultaneous collapse of multiple systems.

The objective of this study was to investigate the process of protein aggregation during aging in more detail, especially regarding the question of how the coalescence of protein aggregates declines in its function during aging.

To address this, a misfolding, temperature sensitive allele of Pyrroline-5-carboxylate reductase (PRO3), i.e. pro3-1 was used. We observed that pro3-1 is able to be efficiently incorporated into 1-2 inclusion bodies during heat stress. However, this mutant protein does not efficiently coalesce in aging cells and instead forms a large number of small aggregates, indicating a failure of specific protein quality control pathways. Using an SGA-based high content microscopy screen of the yeast knockout library we screened for genes that control pro3-1 coalescence after heat shock. We identified genes from membrane coats, tethering factors, vesicle trafficking, cell polarity and protein turnover to be major players of pro3-1 aggregate coalescence after heat shock. Of these however, only genes from TOR (target of rapamycin) signaling showed an impact on pro3-1 aggregate coalescence during aging.

245 Metabolic Cooperation Modulates Eukaryotic Microbial Communities Ageing and Survival.

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The presence of extracellular metabolites aids metabolic interactions occurring frequently at the cell-environment and cell-cell interfaces. Metabolic interactions vastly rely on the exchange of metabolites and can determine fitness and survival of individual cells and overall population, by overcoming metabolic inefficiency between cells. Nevertheless, limited is our understanding of how metabolite exchange operates in eukaryotes, particularly how it is established and how it impacts on cellular fitness over time. Using self-establishing metabolically cooperating communities (SeMeCos), we focused in understanding how metabolic dependencies modulate eukaryotic microbial communities' survival. Coupling the SeMeCo system with chronological lifespan assays, we observed that SeMeCos have increased lifespan compared to wild type yeast and that this is accompanied by increased proportion of consumer versus producer cells. We further identified age-associated metabolic cooperation profiles, i.e. metabolic dependencies that have a significant effect on lifespan. Importantly, intracellular and extracellular metabolic profiling revealed that cells were not deprived of metabolites in SeMeCos cultures, excluding a "dietary restriction-driven lifespan extension phenotype" upon sharing of metabolites. Mechanistically, proteomics analysis identified the GO terms Mitochondria, Redox Stress processes and Metal Ion Binding Proteins as being enriched in SeMeCos. We are currently exploring these processes by performing both oxidative stress resistance assays, to identify associated redox regulating metabolites and underlying pathways, and ionomics analysis, to identify metal ions and associated binding proteins, that underlie the metabolic cooperation driven lifespan extension. Impairment or inability to metabolically interact drives cellular dysfunction, which accompanies ageing and disease, therefore uncovering how cells metabolically interact might aid the development of therapies targeting these processes.

246 Mechanisms Underlying the Distribution of Parental and Newly Synthesized H3-H4 Histones during DNA Replication

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Histone post-translational modifications control DNA accessibility and regulate the interactions between chromatin and other proteins like transcription factors and repressors. Thus, these epigenetic marks establish a landscape across the genome that determines the transcriptional program of each cell. Compromising the accurate distribution of histone modifications not only results in profound alterations in gene expression but also leads to heterochromatin loss and genomic instability. During DNA replication, nucleosomes must be stripped from the DNA to allow the passage of the replication fork and then reassembled on the nascent DNA. To avoid a major perturbation of the chromatin landscape, parental histones, carrying the epigenetic information, must be deposited close to their original positions and evenly distributed between the leading and lagging DNA strands. To study this process, we use the enrichment and sequencing of protein-associated nascent DNA (eSPAN) method which allows us to quantify the relative abundance of a protein between the leading and lagging strands on the nascent DNA. Using H3K4me3 and H3K56ac as representatives marks of parental and newly synthesized histones, respectively, we have observed how parental and new nucleosomes are segregated almost symmetrically between both nascent DNA strands. This is orchestrated by several replication factors involved in shuttling parental H3-H4 histones to the leading or lagging strand. Deletion of these factors or mutations at their histone-binding domains results in an asymmetrical distribution of parental H3-H4 during replication and a loss heterochromatin silencing. Moreover, by following the distribution of H3K4me3 over time on nascent DNA, we have seen how different genomic regions show different kinetics of reestablishment of this transcription-associated mark. These studies provide a unique insight into the mechanisms of epigenetic inheritance into daughter cells and the maintenance of gene expression programs during DNA replication.

247 The Candida albicans HIR Histone Chaperone Complex is a Novel Player in Fungal Virulence

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The yeast Candida albicans is a component of the normal human gut microbiota, which frequently causes a wide range of infections. The fact that C. albicans is able to thrive both as a gastrointestinal commensal and as a deadly invasive pathogen, reflects the high capability of this fungus to adapt to various host niches with distinct environmental stresses. For instance, C. albicans readily switches between different morphologies such as the unicellular yeast form and the filamentous hyphal growth phase in response to numerous host signals. Such rapid environmental adaptations are governed by dynamic transcriptional changes, which are intertwined with dynamic chromatin re-organization. Key players in chromatin homeostasis are histone chaperones such as the HIR complex, which facilitates chromatin assembly and might act as a transcriptional co-regulator. Therefore, we aimed to characterize the role of the HIR complex in fungal environmental adaptation and thus, virulence. We found that, genetic removal of the HIR complex member Hir1 decreases transcriptional amplitudes in response to hyphal–inducing conditions, which results in decreased sensitivity to hyphal triggers of the HIR1-deletion strain. Additionally, HIR1-deficient cells hyperinduce the transcription of some secreted aspartic proteases (SAPs) in response to protein as the main nitrogen source. Consequently, the HIR1 mutant shows accelerated growth and a dramatic gain in fitness under these conditions. Using epistasis analysis, we provide evidence that Hir1 affects gene transcription downstream through the SPS sensing pathway, using the associated transcription factor Stp1, which is essential for the assimilation of nitrogen from proteins. Since nutrient acquisition from alternative sources is crucial for pathogen fitness, we hypothesized that the loss of HIR1 affects fungal virulence. Indeed, in a mouse model of systemic infection, hir1Δ/Δ cells display a dramatic hypervirulence phenotype with increased fungal kidney burdens. These data indicate that loss of HIR1 provides a fungal growth advantage in vivo leading to enhanced virulence. To further uncover how the disruption of the HIR complex alters fungal chromatin architecture, and thus transcriptional fine-tuning, we have used ATAC-seq to reveal how HIR affects the functional chromatin landscapes during different growth conditions. In summary, this work demonstrates a novel link between replication-independent chromatin assembly and virulence in a human fungal pathogen.

248 Molecular Complex Data at the Saccharomyces Genome Database

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Proteins seldom function individually. Instead, they interact with other proteins or nucleic acids to form stable macromolecular complexes that play key roles in important cellular processes and pathways. One of the goals of the Saccharomyces Genome Database (SGD; www.yeastgenome.org) is to provide a complete picture of budding yeast biological processes. To this end, we have collaborated with the Molecular Interactions team that provides the Complex Portal database at EMBL-EBI to manually curate the complete yeast complexome. These data, from a total of 589 complexes, were previously available only in SGD's YeastMine data warehouse (yeastmine.yeastgenome.org) and the Complex Portal (www.ebi.ac.uk/complexportal). We have now incorporated these macromolecular complex data into the SGD core database and designed complex-specific reports to make these data easily available to researchers. These web pages contain referenced summaries focused on the composition and function of individual complexes. In addition, detailed information about how subunits interact within the complex, their stoichiometry, and the physical structure are displayed when such information is available. Finally, we generate network diagrams displaying subunits and Gene Ontology (GO) annotations that are shared between complexes. Information on macromolecular complexes will continue to be updated in collaboration with the Complex Portal team and curated as more data become available.

249 Evidence for Separation of PRPP Production and Maintenance of Cell Wall Integrity: Two Essential Functions of the PRPP-synthesising Machinery in Saccharomyces cerevisiae.

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At least 10% of the 6000 genes that make up the genome of Saccharomyces cerevisiae are duplicated. We have identified a family of five PRS genes, each capable of encoding PRPP (phosphoribosyl-pyrophophate) synthetase. The sequence similarity between yeast and human PRS genes has allowed us to create genocopies of the mutations associated with altered Prs activity and examine their effect on yeast physiology. Lithium, a natural Gsk3 inhibitor and a mood stabiliser for the treatment of bipolar disorder inhibits the growth of yeast when PRS1, PRS3 or PRS5 have been deleted indicating an involvement of Prs in neuropathology and cognitive deficits of central nervous system disorders, e. g. Charcot Marie Tooth disease (CMTX5). Prs5 is unusual that it is one of the 11 triply phosphorylated proteins in yeast. Rim11, one of the four Gsk3 paralogous proteins was verified as a partner of Prs5. When the three phosphorylatable S364, S367 and S369 were mutated or deleted the Prs5-Rim11 interaction was reduced by 70% supporting the role of Rim11 as a kinase for posttranslational modification of the three phosphosites in Prs5. This is further supported since Prs5 contains a priming site C-terminal to the site of Gsk3 phosphorylation, a prerequisite of Gsk3 for targeting proteins. Mutation of the three phosphosites of Prs5 impinges on the expression of the transcription factor Rlm1, an endpoint of the cell wall integrity (CWI) pathway. The loss of the heterodimer Prs1/Prs3 also leads to impaired CWI since both Prs1 and Prs3 interact with Rlm1. The phosphorylated MAP kinase Mpk1/Slt2 of the CWI pathway interacts with Prs1 as shown by immunoprecipitation. In the absence of Prs3, Prs1 is unstable. Prs3 interacts with the kinetochore-associated protein, Nuf2. Prs3 contains a nuclear localisation site whose loss causes caffeine sensitivity and reduction in Rlm1 expression in response to Mpk1/Slt2 activation. Therefore, the synthetic lethality caused by the loss of Prs3 and Prs5 is in fact due to the loss of the Prs1/Prs3 functional subunit and is in agreement with three of the five Prs proteins being required to correct CWI signalling by nucleo-cytoplasmic shuttling. We will discuss that the two metabolic functions of Prs, provision of PRPP and maintenance of CWI, is an example of division of labour.

250 Exploring Saccharomyces Cerevisiae As A Production Platform For Biopharmaceuticals.

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In today's society recombinant protein production for biopharmaceutical applications has become a billion dollar industry. This is mainly due to the superiority of biopharmaceuticals in activity, efficiency, and specificity towards their biological targets over conventional small molecule pharmaceuticals. One important cell factory is the extensively studied budding yeast, S. cerevisiae, which is known for the commercial production of several large volume products including insulin.

In our work, we compare two strategies for developing a S. cerevisiae strain that can be used as a platform for multiple recombinant protein production and secretion. This involved strain generation either through random UV-mutagenesis or by targeted engineering. Moreover, the pharmaceutical proteins we selected differ deliberately in size and complexity in order to test the production potential of the two engineered S. cerevisiae strains we constructed.

Our experiments have shown that We have the strains differ in their capability to produce certain proteins. The strain made by UV-mutagenesis shows to produce and secrete all proteins of different sizes and complexity where the strain generated by targeted engineering can produce some of the protein. At this moment we cannot formulate an exact hypothesis on the methodology for the distinction between the secreted and non-secreted proteins but we are looking into the relation of N-glycosylation sites and recombinant protein production and secretion.

251 Hexose and Glycerol Metabolism in the Yeast Yarrowia lipolytica.

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The non-conventional yeast Yarrowia lipolytica utilizes numerous carbon sources including hexoses and sugar alcohols such as glycerol. In contrast to the model yeast Saccharomyces cerevisiae, Y. lipolytica preferentially uses glycerol over hexoses, a phenomenon that remains to be explained. The RNA-Seq data from cells growing under chemostat conditions in either glycerol, glucose, fructose or mixture of glycerol and glucose show that there are no observable differences in hexokinase (HXK1) expression levels between the experimental conditions but the genes responsible for substrate-specific transport across the cell membrane, specifically YHT1 and YHT4 hexose transporters, are more highly expressed in presence of glucose and fructose than glycerol. Conserved motif search in the promoter regions of genes upregulated by hexoses and/or downregulated by glycerol, namely sugar transporters and POX genes, revealed that expression of these might be controlled by a signaling cascade with Snf1 kinase involved. Since Y. lipolytica does not show presence of typical sugar sensors but only sensor-like sugar transporters with 50 amino acid cytoplasmic regions, we hypothesize that this microorganism probably regulates hexose uptake only by one carbon source-dependent regulatory pathway - Snf1 dependent. The preferential uptake of glycerol still remains to be explained.

Furthermore, preferential uptake of glucose over fructose, a phenomenon observed also in the yeast S. cerevisiae, is caused by the native Y. lipolytica hexokinase affinity towards glucose. In the experiments with Y. lipolytica mutants overexpressing heterologous fructophilic hexokinase from Schizosaccharomyces pombe (SpHxk1) fructose and glucose were utilized simultaneously.

Understanding of the complex regulatory mechanism behind hexoses and glycerol utilization will allow to design controllable and efficient biotechnological processes with Y. lipolytica using waste lignocellulosic biomass as well as crude glycerol as cost effective carbon sources.

252 Loss of Physicochemical Homeostasis in Yeast Replicative Ageing.

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Ageing is a process of progressive decline in homeostatic capacity. While a lot of research has been dedicated to investigating molecular gatekeepers of cellular homeostasis, little is known about the physicochemical aspects of cell functionality during ageing. It already has been hypothesized that age-associated changes of parameters such as crowding and pH can drive loss of intracellular organization (Alberti and Hyman, 2016). Here, we present a system to follow physical and chemical parameters in yeast replicative ageing based on the combination of biosensors and microfluidics. We characterized the three biggest cellular compartments (vacuole, cytosol and nucleus) during yeast replicative ageing, and focused on macromolecular crowding, pH and volume, as these three parameters have been previously proposed to influence age-related phenotypes, such as protein aggregation, division frequency and lifespan. Our data shows that loss of physicochemical homeostasis is a cellular ageing phenotype that contributes to a crisis in cell compartment identity, and provides a new context for the established hallmarks of ageing.

253 The Protein Kinase HRK1 Enhances Glucose Uptake Through the Hexose Transporter Hxt1 in Saccharomyces cerevisiae.

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Saccharomyces cerevisiae HRK1 gene encodes a Ser/Thr protein kinase of poorly known function belonging to the "Npr1-family" proposed to regulate plasma membrane transporters activity. Hrk1 is one of the most important determinants of tolerance to acetic acid stress in yeast being one of the gene targets of the transcription activator Haa1 (1). Haa1 is the main player controlling the transcriptional activation of genes involved in the response and tolerance to acetic acid in S. cerevisiae (1) and in the highly acetic acid tolerant food spoiler yeast Zygosaccharomyces bailii (2, 3, 4).

The first biological role attributed to Hrk1 was the activation of the plasma membrane proton pump Pma1. A quantitative phosphoproteomics analysis of membrane-associated proteins showed that HRK1 deletion impacts the membrane-associated phosphoproteome in acetic acid-stressed yeast cells and in cells under non-stressful conditions (5) Hxt1 was among the membrane proteins whose phosphorylation levels were altered upon deletion of HRK1 (5). Hxt1 is a low-affinity, high-capacity hexose transporter activated in conditions of saturating extracellular glucose concentrations (1% (w/v)) and inhibited at concentrations below 0.1% (w/v) (6). The influence of HRK1 expression on Hxt1 was assessed by comparing the glucose uptake kinetic properties of Hxt1 and the fermentation performance of the single hexose transporter expression strain HXT1+ (7), either or not expressing HRK1. Results indicate that Hrk1 plays a role in the activation of glucose uptake and fermentation mediated by Hxt1, providing useful information to guide the development of superior industrial yeast strains with improved fermentation performance in particular of acetic acid-rich- biomass residues hydrolysates to be used in biorefinery.

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254 Yeast-Human Cross-Species Complementation and Associations with Disease-related Genes.

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The Saccharomyces Genome Database (SGD; http://www.yeastgenome.org) is a comprehensive resource for curated, molecular and genetic information on the genes and proteins of S. cerevisiae. Model organism genetics holds great promise for advancing our understanding of human gene function and involvement in disease. Elucidating the biology of yeast genes has in many cases provided valuable insight into the function of their homologous human counterparts. With the goal of making connections between yeast genes, their human homologs and associated diseases, we have undertaken a project to collect and display this information at SGD.

At the start of this project, yeast-human cross-species functional complementation results were collected from the literature and stored in the YeastMine data warehouse where the data can be accessed using preformed template queries. Relevant information was also added to the respective Locus Summary Page descriptions. These functional complementation relationships including some where genes also share homology and the corresponding relationship types have been stored in the database and are now displayed on SGD pages. A subset of these human homologs have been determined to be disease associated. For this subset, the corresponding disease ontology (DO) terms were identified and associated with both the human gene and the corresponding yeast homolog, along with supporting information. Diseases associated with human genes that have a computationally determined yeast homolog have also been included in this set. Disease pages have been designed that include the following pieces of information: disease name, ID and definition from DO, yeast systematic and ORF names, human HGNC-approved gene names (https://www.genenames.org), annotation type (manual vs HTP), evidence code, reference, source and relevant links. A disease summary that has been generated at SGD is included on relevant Locus Summary pages with a link to the browsable Disease page. It is our hope that making this information available to our users will facilitate studies aimed at understanding the biological functions of these genes and the role these genes play in the pathology of disease.

255 Regulation of Ceramide Synthase and CK2 by Calcineurin During ER stress.

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ER stress is associated with accelerated aging and degeneration in humans and in yeast. In yeast, multiple signaling pathways become activated in response to ER stress, including the UPR which serves to promote the repair of damage and desensitization to stressors. Activation of the calcium signaling pathway involving calcineurin promotes long-term survival of stressed cells, but how this occurs has remained mysterious. We performed phospho-proteomic analyses of stressed yeast cells at 5, 15, and 45 minutes following addition of FK506, a potent inhibitor of the protein phosphatase activity of calcineurin. Approximately 30 proteins exhibited robustly increased levels of phosphorylation at the earliest time point, and most of the phosphorylation sites were known or consensus targets of the protein kinase CK2. Using western blots, phosphorylation of the CK2 substrate Efb1 declined in response to ER stress and rapidly recovered in response to FK506. Using in vitro kinase assays with crude cell lysates, CK2 kinase activity in stressed also declined and increased rapidly and transiently upon FK506 addition. This effect was dependent on the Cka2 catalytic subunit of CK2 but not the semi-redundant Cka1 subunit. Survival of cka2Δ mutant cells, but not cka1Δ mutant cells, during exposure to ER stress and FK506 was strongly increased. These findings suggest calcineurin may promote survival through negative regulation of CK2, which previously had been thought to be constitutively active. Of many known substrates of CK2, the redundant catalytic subunits of ceramide synthase (Lag1, Lac1) and another regulator of ceramide synthesis in the ER (Svf1) were all found to be important mediators of the cell death triggered by ER stress and FK506. Phospho-mimetic mutations in Lac1 and Lag1 at the CK2 phosphorylation sites were combined with knockout mutations in Svf1 and Cka2 abolished control of the process by calcineurin. Aureobasidin A, which increases ceramide by blocking its conversion to sphingolipid in the Golgi complex, triggers rapid cell death that can be slowed by inhibitors of ceramide synthase and slowed in cka2Δ mutants. Together, these findings suggest that calcineurin promotes survival of stressed cells mainly by restricting CK2-dependent production of ceramide, which could become toxic in cells that have defects in glycoprotein or sterol biosynthesis in ER.

256 An a priori Model for Yeast Cell Expansion During Vegetative Growth.

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Morphogenesis, is a central question in biology and is of particular importance for a comprehensive understanding of model organisms, such as Saccharomyces cerevisiae. While cellular growth of yeast is well described at the population level, the growth and of single cells is subject to recent research. Considering nutrient uptake, osmo-homeostasis, water flow and cell wall mechanics, we formulated an a priori model for the growth of a single yeast cell, from the expansion a new born daughter cell to the combined growth of mother and bud. This biophysical model consists of a system of highly interlinked ordinary differential equations for the thermodynamic quantities: turgor pressure, osmolarity and cellular volume, integrating concepts from rheology and thin shell theory. The model reproduces cell size dynamics during single-cell growth, budding, and hyper- or hypoosmotic stress and predicts that single-cell growth rate and final size are primarily governed by osmolyte uptake and consumption. Further, the model predicts that bud expansion requires different mechanical properties of mother and bud cell wall. Utilizing AFM-based multiparametric imaging, we show that the elasticity of the cell wall does not differ between both cell compartments and propose that the visco-plastic properties are the distinguishing features instead. Constraining the model with two independent data sets of single-cell volume trajectories, obtained from light microscopic images, provided further estimations on the visco-plastic cell wall properties as well as on other key growth parameters, such as the total osmolyte uptake rate per surface area. Based on first principles the model provides a more accurate description of size dynamics than previous attempts and its analytical simplification allows for easy combination with models for other cell processes.

257 Proteins Evolve on the Edge of Supramolecular Self-assembly.

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About 50% of the proteins of known structure are forming symmetric complexes. Symmetry confers unique geometric and functional properties, but it also poses a risk. In sickle cell disease, the symmetry of hemoglobin exacerbates the effect of a mutation, triggering its assembly into harmful fibrils. We examined the universality of this mechanism and its relation to protein structure geometry. We introduced point mutations solely to increase the surface hydrophobicity in 12 symmetric complexes. Remarkably, all of them responded by forming supramolecular assemblies in vitro and in vivo upon their expression in S. cerevisiae. We distinguish these assemblies from aggregates because proteins retain a folded structure within them. To mark this difference, we call them “agglomerates”. This new type of in vivo assemblies appears to form readily during evolution, prompting us to investigate how cells interact with them. To this aim we carried out systematic colocalization experiments between yeast >70 yeast chaperones and 12 distinct agglomerates. We also evaluated whether specific knock out backgrounds sensitize cells to the formation of such agglomerates. Through these experiments we explore interaction networks connecting these supramolecular assemblies to the cell protein machinery.

258 Candida albicans glutamate dehydrogenase (GDH2) catalyzes environmental alkalization but is dispensable for survival and escape from macrophages

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Candida albicans cells depend on amino acid catabolism to obtain energy for growth and to induce hyphal formation inside phagosomes of engulfing macrophages. It has been proposed that deamination of amino acids by fungal cells is important for neutralizing the acidic microenvironment of phagosomes, and that alkalization provides the stimulus for hyphal growth. Here, we show that mitochondrial-localized NAD+-dependent glutamate dehydrogenase (GDH2), which catalyzes the deamination of glutamate to α-ketoglutarate, is responsible for the observed alkalization of media when C. albicans cells are grown with amino acids as the source of carbon and nitrogen. Using CRISPR/Cas9 we have created a strain lacking GDH2 (gdh2-/-). This strain is viable and does not extrude ammonia on amino acid-based media. Consistent with this finding, but in contrast to current dogma, C. albicans strains carrying inactivated alleles of DUR1,2, encoding cytosolic urea amidolyase, remain competent to alkalinize the extracellular milieu. Furthermore, we confirm that environmental alkalization by wildtype strains does not occur under conditions of high glucose (2%), a finding attributable to glucose-repression of GDH2 expression and mitochondrial function. Inhibition of mitochondrial translation or oxidative phosphorylation by chloramphenicol or Antimycin A, respectively, prevents alkalization. GDH2 expression and mitochondrial function become derepressed as glucose levels are lowered from 2% (110 mM) to 0.2% (11 mM), or when glycerol is used as carbon source. At 0.2% glucose, wildtype strains, but not gdh2-/- mutants, efficiently alkalinize the media. Unexpectedly, inactivation of GDH2 does not affect the induction of filamentous growth of C. albicans, either in vitro in filament-inducing media, or importantly, in situ in the phagosome of primary murine macrophages. Using time-lapse microscopy, we have observed that gdh2-/- cells survive, filament and escape from macrophages at rates indistinguishable from wildtype. These results indicate that alkalization of the macrophage phagosome is not essential for C. albicans survival nor does it provide the morphogenic signal inducing hyphal growth.

259 Proteome-wide Signatures of Function in Highly Diverged Intrinsically Disordered Regions.

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Intrinsically disordered regions make up a large part of the proteome, but the sequence-to-function relationship in these regions is poorly understood, in part because the primary amino acid sequences of these regions are poorly conserved in alignments. Here we use a comparative evolutionary approach to detect molecular features that are preserved in the amino acid sequences of orthologous intrinsically disordered regions. We find that most disordered regions contain multiple molecular features that are preserved, and we define these as “evolutionary signatures” of disordered regions. We demonstrate that intrinsically disordered regions with similar evolutionary signatures can rescue function in vivo, and that groups of intrinsically disordered regions with similar evolutionary signatures are strongly enriched for functional annotations and phenotypes. We propose that evolutionary signatures can be used to predict function for many disordered regions from their amino acid sequences.

260 Identifying Driver Genes Responsible for Condition Dependent Fitness Effects of Synthetic Chromosome Amplifications.

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Whole chromosome aneuploidy and large segmental copy number variants (CNVs) can have devastating effects in multicellular organisms, from developmental disorders and miscarriage to cancer. Aneuploidy in single celled organisms such as yeast also results in proliferative defects and reduced viability. Yet, paradoxically, CNVs are routinely observed in laboratory evolution experiments with microbes in stressful growth conditions. The defects associated with aneuploidy are often attributed to the imbalance of many differentially expressed genes on the affected chromosomes, with many genes each contributing incremental effects. An alternate hypothesis is that a small number of individual genes are large effect 'drivers' of these fitness changes when present in an altered copy number. To test these two views, we have engineered a library of strains with ~2000 synthetic chromosome arm amplifications tiled across the genome such that each amplification extends from a barcoded genomic location to the telomere on the same arm, and neighboring amplifications differ by only a few genes on average. We have competed these strains in nutrient limitation, a condition known to select for aneuploidies, as well as in high temperature, extended stationary phase, and treatment with radicicol – conditions tolerated poorly by disomic yeast. To distinguish between our two hypotheses, we have compared the fit quality of piecewise constant and linear models to the relative fitness data along each chromosome arm. In nutrient limitation and extended stationary phase we find that most chromosomes are better modeled as stepwise patterns, suggesting that individual genes disproportionately drive fitness effects in these conditions. Using these models, we have identified step points for further validation where individual genes may be driving the fitness differences between amplifications. These candidate step points include both regions containing expected driver genes of large effect, such as the multidrug transporter PDR5 in radicicol treatment, and regions without an obvious driver gene, indicating that some drivers may be dependent on the context of a large amplification. To test this possibility, we are validating candidate genes in euploid cells as well as in the presence of the large chromosomal amplification.

261 The Chromatin Remodeler Isw1 Is A Novel Actor Of The Unfolded Protein Response.

Anna Babour and Laura Matabishi

INSERM

Isw1 is the catalytic subunit of the yeast ATP-dependent chromatin remodeling complex ISWI, and is responsible for regular nucleosome spacing along the chromatin fiber. In vivo, inactivation of ISW1 has no effect on cell growth under normal conditions and only a very modest effect on transcription. Hence, although chromatin dynamics is recognized as playing an essential role in the control of DNA-associated processes such as transcription, the physiological role of Isw1 has remained elusive. Recently, a number of studies reported that chromatin dynamics can modulate gene expression by influencing mRNP formation and fate. In this context, we described an unanticipated function for ISWI in the control of gene expression, beyond the transcription process per se. Isw1 acts as a mRNP nuclear export surveillance factor that retains export-incompetent transcripts in proximity of their transcription site. Isw1 directly interacts with mRNA and its quality control activity is achieved via its increased interaction with both faulty RNAs and Rrp6, a catalytic subunit of the nuclear exosome. We proposed that the concerted action of Isw1 and Rrp6 represents a surveillance mechanism for efficiency and accuracy of mRNA biogenesis.

Although this novel function of Isw1/Rrp6 was evidenced in mutants defective for mRNA export, Isw1 interacts with mRNA in a wild-type context. We thus hypothesized that Isw1 operates in WT cells to regulate the subcellular localization (nuclear vs cytoplasmic) of specific transcripts and thereby fine-tunes gene expression. Consistently, a genome-wide analysis of transcripts that directly bind Isw1 in vivo identified the HAC1 mRNA as a bona fide Isw1 target. HAC1 encodes the transcription factor of the Unfolded Protein Response (UPR), an adaptive response to endoplasmic reticulum (ER) stress that is set in motion when the demand for protein folding outcompetes the capacity of the organelle The UPR is initiated by the unconventional excision of the translation-inhibitory intron of HAC1 in the cytoplasm, that is in turn translated into the Hac1 transcription factor. We show that Isw1 is a novel actor of the UPR, which, by controlling the subcellular localization of the HAC1 transcript, profoundly affects the UPR output.

262 The Use of the Gene Ontology to Describe Biological Function at SGD.

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The Saccharomyces Genome Database (SGD; www.yeastgenome.org) is a comprehensive resource of curated molecular and genetic information on the genes and proteins of Saccharomyces cerevisiae. Since 2001, SGD has used the Gene Ontology (GO) to annotate the functions of gene products in budding yeast. The GO comprises three sets of structured, controlled vocabularies, or “ontologies”: the Molecular Function ontology describes activities of gene products; the Biological Process ontology places these molecular functions in a biological context; and the Cellular Component ontology indicates the subcellular localizations of gene products. Expert curators select GO terms to apply to gene products based on published scientific literature. At SGD, results from traditional experimental methods are the primary sources of evidence used to support GO annotations. In addition, results from comparative sequence and genomic studies, as well as analyses of functional genomic and proteomic data, have provided valuable insights into the biological roles of gene products, and these data are incorporated into SGD as well. SGD has several web interfaces and analysis tools that display and use these data. The Locus Summary lists each manually curated and high-throughput GO annotation and indicates when computational GO annotations are available. The GO Term Finder searches for significant shared GO terms used to describe the genes in an input list to aid in discovery of potential gene similarities. The GO Slim Mapper maps annotations of a group of genes to more general terms and/or bins them into broad categories, also known as “GO Slim” terms. Gene Ontology annotations are also incorporated into YeastMine, SGD’s multifaceted search and retrieval environment that provides access to diverse data types. Searches can be initiated with a list of genes or a list of Gene Ontology terms. These interfaces and tools are important as part of SGD's ongoing mission facilitate research, education, and discovery using the Gene Ontology.

263 Genetic profiling of protein burden and nuclear export overload

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Ultimate overproduction of a gratuitous protein causes growth defect by overloading cellular resources for protein production. This phenomenon is called the protein burden. Despite that the protein burden itself seems to be a simple phenomenon at a glance, little is known about the physiological conditions and cellular responses triggered by the protein burden.

Here, we surveyed genetic interactions between mutant strains and the strong GFP overproduction (GFP-op) in S. cerevisiae. To isolate confident mutant sets showing positive- and negative- genetic interactions with the protein burden; we used a condition to cause remarkable growth defects by strong GFP-op from the TDH3 promoter on a multicopy plasmid; we surveyed temperature-sensitive collections of essential genes in addition to the deletion collection of non-essential genes; we performed strict statistical evaluation to isolate mutants showing genetic interactions. We also tried to genetically distinguish the protein burden and other process overloads by surveying genetic interactions between those mutant strains and overexpression of a GFP with nuclear export signal (NES-tGFP).

As a result, we identified several cellular processes specifically interacting with the protein burden; the polarization process showed negative interaction, while the RNA degradation and the general transcriptional control showed positive interactions. We found a large, tandemly-repeated protein like the tGFP induced overload of the proteasome. We also revealed potential factors determining the capacity of the nuclear export, and major essential cargos transported by this process.

264 Efficient ER Exit of Mnn4 Is Dependent on Both Svp26 and Mnn6 in Saccharomyces cerevisiae.

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Incorporation of newly synthesized membrane proteins into COPII vesicles is facilitated either by direct association of cargo proteins with COPII coat proteins or by ER exit adaptor proteins which mediate the interaction of cargo proteins with COPII coat proteins. Svp26 is one of such ER exit adaptor proteins in yeast Saccharomyces cerevisiae. ER exit of several type II membrane proteins It was reported that the ER exit of several type II membrane proteins were facilitated by Svp26. We present here that Mnn4, a type II membrane protein required for mannosyl phosphate transfer to glycoprotein-linked oligosaccharides, is localized to the Golgi and its efficient incorporation into the COPII vesicles is also dependent on Svp26. In addition to Mnn4, Mnn6 is known to be also required for transfer of mannosyl phosphate to the glycans. We show that Mnn6 localizes to the ER by indirect immunofluorescence. As in the case with Svp26, deletion of the MNN6 gene results in the accumulation of Mnn4 in ER. In vitro COPII vesicle budding assays show that Svp26 and Mnn6 facilitate the incorporation of Mnn4 into COPII vesicles. In contrast to Svp26, which is itself efficiently incorporated into the COPII vesicles, the incorporation of Mnn6 into the COPII vesicles was very low. Both Mnn4 and Mnn6 have the DXD motif that coordinates a divalent cation essential for the glycosylation and is hence often found in the many glycosyltransferases. Alcian blue dye binding assay show that substitution of the first D in this motif present in Mnn4 by A compromises the Mnn4 function. In contrast, amino acid substitutions in DXD motifs in Mnn6 did not affect the function of Mnn6. These results suggest that Mnn4 may be directly involved in the mannosy phosphate transfer reaction.

265 Insights into protein production bottlenecks in Pichia pastoris: complexity of expressing thermostable phytases

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Over the last three decades, Pichia pastoris (Komagataella phaffii) has become an important platform for heterologous protein production. High cell density, simple growth media and high secretory capabilities for recombinant proteins, while secreting low amounts of endogenous proteins, make this yeast a successful microbial cell factory. Recent developments in synthetic biology have extended the tool box for genetic engineering of P. pastoris to improve production strains. New molecular tools include synthetic promoters for fine-tuning of expression, glyco-engineered strains to mimic human glycol-forms and CRISPR/Cas9 technology available for genomic engineering. Several enzymes are commercially produced in P. pastoris, with phytases being one of the biggest on the global market with sales in excess of $1 billion. Phytases are ubiquitously used as dietary supplement for swine and poultry to increase digestibility of phytic acid, the main form of phosphorous storage in grains. Use of phytase reduces the addition of inorganic phosphate to diets and decreases the anti-nutritional effects of phytate. Significant effort has been directed towards producing improved enzymes with higher activity, increased stability and at economic levels in industrial fermentations. As such, there are excellent products on the market, but there is a continuing demand for further improvements to drive down costs and for enzyme manufacturers to increase market share. One disadvantage of phytase is an inability to withstand the high temperatures required during industrial steam pelleting. Hence, protein engineering to develop thermostable phytases is a key research focus. Common modifications to improve thermostability include introduction of disulfide bonds and glycosylation sites, which in turn increase folding complexity and decrease cellular production. Genetic engineering of strains to improve expression of complex protein structures is therefore crucial to achieve cost-effective industrial manufacture. In this work, potential bottlenecks for expression of E. coli AppA phytase and thermostable variants in P. pastoris were explored. Recently developed bidirectional promoters were tested for expression of the phytases together with HAC1, a transcriptional regulator of the unfolded protein response. Further co-expression of phytases with folding chaperones, disulfide bond isomerases, vesicle transport proteins and a cytosolic redox metabolism protein are presented, bringing insights into recombinant protein folding and secretion in this important expression platform.

266 The Transcription Factor Sfp1 Regulates the Oxidative Stress Response in Candida albicans

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Because of the increasing immunocompromised population and the limited classes of antifungal drugs available, Candida albicans has emerged as an important opportunistic pathogen with high mortality rates. During infection and therapy, C. albicans frequently encounters immune cells and antifungal drugs, many of which exert their antimicrobial activity by inducing the production of reactive oxygen species (ROS). Therefore, antioxidative capacity is important for the survival and pathogenesis of C.albicans. In this study, we characterized the roles of the zinc finger transcription factor Sfp1 in the oxidative stress response against C. albicans. A sfp1-deleted mutant was more resistant to oxidants and macrophage killing than wild-type C. albicans and processed an active oxidative stress response with the phosphorylation of the MAPK Hog1 and high CAP1 expression. Moreover, the sfp1-deleted mutant exhibited high expression levels of antioxidant genes in response to oxidative stress, resulting in a higher total antioxidant capacity, glutathione content, and glutathione peroxidase and superoxide dismutase enzyme activity than the wild-type C. albicans. Finally, the sfp1-deleted mutant was resistant to macrophage killing and ROS-generating antifungal drugs. Together, our findings provide a new understanding of the complex regulatory machinery in the C. albicans oxidative stress response.

267 Novel Insights in the Mode of Action of Diverse Antifungal Plant Defensins from High Throughput Screens of the S. cerevisiae Non-Essential Gene Deletion Collection

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Onychomycosis is a fungal nail infection with a worldwide prevalence of 5.5%. The result is painful nails, occupational hindrance as well as psychological associated affects. Onychomycosis is increasingly prevalent due to an aging population, a rise in the number of immunocompromised patients and an increase in physical activity for fitness. Current therapies have limited success rates largely due to nail penetration by antifungal drugs. Using our lead antifungal we have demonstrated that plant defensins penetrate nails well and as such are attractive options for new antifungal treatments. Plant defensins share a similar three-dimensional structure but vary significantly in sequence. This sequence variability accounts for the differences in their mechanism of action.

Elucidating the mechanism of action of individual defensins using current methods is very time consuming and may miss critical aspects of the mechanism. We have screened the

S. cerevisiae non-essential gene deletion collection using next generation sequencing (NGS) to identify genes that provide for a differential response to defensins. This allows for detailed information on the mechanism of action for up to five defensins to be collected simultaneously.

Each deletion strain contains a unique barcode for identification. After treatment with defensins, NGS was used to identify the relative abundance of each barcode in the pool. These counts identified strains resistant to each defensin. Bioinformatic analysis of the results revealed enrichment of genes in processes relating to key organelles such as the mitochondria and the vacuole. These patterns of enrichment differed for each of the five defensins, indicating five unique mechanisms of action in the defensins tested. We have used antifungal assays and microscopy with cell process-specific dyes to validate the mechanisms identified in the screen.

This data indicates that high-throughput sequencing of a yeast deletion library is a useful technique to rapidly identify antifungal molecules with novel mechanisms of action, as well as molecular detail about the mechanisms of action. Some fungal responses observed have not been reported for antifungal plant defensins and represent a significant step-forward in the field.

268 Evaluation of Yeast as a Production Host for Novel Capsaicinoids.

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The pungency of the chilli pepper fruit comes from alkaloid molecules called capsaicinoids, which besides spicing up foods, also have been reported to possess several pharmacological effects. They are, for example, strong agonists of TRPV1 (vanilloid receptor 1), which is an important drug target for the treatment of pain disorders. Capsaicinoids are produced in the chilli pepper plant from the precursors vanillylamine and a medium chain acyl-CoA. Acyl-CoA substrates can be saturated or unsaturated, branched or unbranched, and have different chain lengths, thereby resulting in different capsaicinoids depending on the availability in plantae. Currently, capsaicinoids are manufactured by extraction directly from the chilli pepper plant, which can cause several inconsistencies depending on the season and amount. Another possible strategy to generate capsaicinoids is to transfer the required biosynthetic pathways to a microbial production host. In this project, we are evaluating the potential of baker's yeast Saccharomyces cerevisiae as a production system for novel and structurally diverse capsaicinoids with potentially improved bioactivity. Data on the evaluation of engineered yeast will be presented.

269 Yeast8: The new version of consensus yeast GEM developed in a community way

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The first yeast genome scale metabolic model (GEM) named 'iFF708' was released in 2003. After that, tens of different versions of yeast GEM for S. cerevisae were put forward by different research groups from worldwide. Among them, the consensus yeast GEM series from Yeast1 to Yeast7 were developed from 2008 to 2013. These metabolic models represent a comprehensive strain-specific database for yeast metabolism, thus being widely used in system biology and metabolic engineering. As new omics data from phenotype to genotype continuously appear, it becomes essential to maintain the consistent upgrade of yeast GEM so that it could meet the strong demand from the wide community in big omics data analysis and design-construction-evaluation-optimization (DCEO) biotechnology. Here, we developed the consensus yeast GEM in a community way and continuously improved the version from Yeast7.6 (3493 reactions) to Yeast8.3 (3963 reactions). During the process, not only the model size and quality were improved significantly, but also every new change in the model curation, including the newly added physiological data, gene annotation data, scripts, et al., were recorded and stored systematically in our repository-https://github.com/SysBioChalmers/yeast-GEM. This repository is free and public, so everyone could have an easy access for the latest version of yeast GEM. Meanwhile, any new feedback from the community could be conveniently recorded and later used for the new improvement of yeast GEM. Therefore, as a new standard, We hope that the Yeast8 and related repository could contribute to the research in system and synthetic biology of yeast for wide community.

270 Metabolic modeling of the Candida species

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Candida species are among the most common human fungal infections, which cause both mucosal and deep tissue infections. Recent evidences indicated that the metabolic versatility and the adaptation to host microenvironment of fungal pathogens affect host-fungus interactions during the infection and effect on the fungal susceptibility to anti-fungal drugs. Systems biology approaches has greatly contributed toward the understanding of disease and host-pathogen interactions during infection. Genome-scale metabolic modeling, had been employed for the analysis of pathogen infections (e.g., Mycobacterium tuberculosis, Neisseria meningitides, Plasmodium falciparum), and for the discovery of novel drug targets suitable for treating the infections or diseases. In this study, published genomics, transcriptomics (RNA-seq or microarray) and phenomics data for sequenced Candida species were collected based on literature mining. Enzymes from 12 Candida species and 2 reference species were annotated using a self-developed pipeline, and were used to reconstruct genome-scale metabolic models. A probability based major voting algorithm was developed to integrate orthologous information and enzyme annotations together to reduce the false-positive rate of enzyme annotation. Phylogenetic analysis of metabolic gene distributions reveal similar pattern as the phylogenetic analysis of marker genes, which revealed that only subtle differences in metabolism are observed between different Candida species. Interestingly, significant differences were found in glycan metabolism, secondary metabolism or other amino acid metabolism. Moreover, analysis of enzyme expansion rates also showed that enzymes involved in carbohydrate and amino acid metabolism have significantly faster expansion rates. All together, these results indicated that the heterogeneity and plasticity of metabolism in Candida species may have evolved to respond to specific sets of host environmental cues.

271 Engineering of the Central Carbon Metabolism to Produce Fatty Acid-Derived Compounds in Saccharomyces cerevisiae.

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Chalmers University of Technology

Fatty acids and compounds derived thereof have a wide variety of industrial applications, e.g. as fuels, food ingredients, cosmetics and pharmaceuticals. S. cerevisiae does not naturally accumulate substantial amounts of fatty acids. In order to transform it into a platform organism for the biotechnological production of fatty acid derived chemicals, major modifications of its central metabolism are required.

Acetyl-CoA represents the universal precursor for fatty acids. Different heterologous pathways were expressed in S. cerevisiae that could bypass native pathways and by this provide increased amounts of acetyl-CoA. Such strategies however often have a detrimental effect on growth. Adaptive laboratory evolution was therefore applied to improve the growth rate of the engineered strains. Using systems biology analysis, it was possible to identify the causal mutations responsible for the improved phenotype.

In addition to the precursor acetyl-CoA, NADPH is needed as a reducing co-factor for fatty acid biosynthesis. In an attempt to increase the flux through the pentose phosphate pathway (PPP), a major source of NADPH, the transcription factor Stb5 was overexpressed. This indeed led to improved fatty acid production during growth on glucose, which was however independent of PPP flux.

272 Quantifying Cellular Responses to Heat-Shock Using Transmission Electron Microscopy of High-Pressure Frozen Yeast Cells

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The presence of established mechanisms to cope with stressors such as environmental changes, starvation or hypoxia is essential for survival in all species on earth. Temperature change is a significant problem for all living organisms as even an only moderate shift above or below optimum growth temperature can dramatically affect survival. Although the response to temperature shifts is well characterized at the molecular level, there is no comprehensive and quantitative model for structural changes within the cell after heat-shock. Understanding how cells deal with environmental stress also helps understand their response to other stressors and how to reduce their impact on the cell. Using transmission electron microscopy of high-pressure frozen yeast cells, we have imaged thousands of cells in different stages of heat-shock and thoroughly examined structural changes. So far, we have quantified the size, number and location of most organelles and some of their contact sites during this heat-shock timeline. We have observed an increase in the volume of cells, mitochondria, vacuoles, lipid droplets, and an enlargement of organelle contact sites in heat-shocked cells. Such changes possibly indicate an arrested cell cycle, slowing regeneration and reproduction. An unbiased classification and quantification of structural cellular changes creates new maps of the internal organization of the cell during cellular stress. Unexpected changes in morphology and sizes of organelles, such as the significant increase in volume and number of lipid droplets, can now be further investigated using molecular cell biology and genetics. This can unravel potentially uncharacterized cellular stress response mechanisms or bring new light to already described pathways.

273 Uncovering The Genetics Of Adaptability

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The capacity to adapt to environmental challenges via temporary or permanent changes to the composition of cells is essential to all living organisms. Adaptation can be achieved by plastic, epigenetic or genetic mechanisms. Because the capacity to adapt fast is advantageous in a fluctuating or rapidly changing ecological context, adaptability itself is exposed to second order selection and may be an intrinsic, genetically encoded feature of life. We sought to exhaustively explore the genetic underpinnings of adaptability, by adapting a haploid yeast deletion collection of 4700 gene knockouts to arsenic while tracking fitness at near single generation resolution. Arsenic adaptation across generations is fast, predictable and entirely genetic with amplifications of the extrusion (ACR3) and loss of the import system (FPS1, ASK10) consistently explaining the response. We found adaptation speed variation to be explained predominantly (R=0.74-0.54) by genotypes fitness, with initially unfit genotypes tending to faster compensate for their initial defects. This was not due to fit genotypes reaching a selection plateau set by the intrinsic limitations on yeast growth but rather reflected a continuous slowdown as they approached this state. We propose that the adaptation slowdown reflects the profound effects of a diminishing fitness return of arsenic adaptive de novo mutations as populations approach the global fitness peak. We identified >600 mutants with aberrant adaptation kinetics, either unfit genotypes, failing to compensate for their defects or genotypes with normal fitness but higher or lower adaptability than normal. We are currently validating and exploring the mechanistic basis of these deviations. We aim to shed light on the biology of adaptation in evolution and reveal targets whose chemical inhibition could prevent or slow down resistance development in cancer and infection.

274 Metabolic engineering of lignocellulosic yeast to co-ferment cellobiose and xylose

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Lignocellulosic biomass offers a renewable alternative to fossil fuels. Glucose and xylose are the major sugars in this biomass which are hydrolysed products of cellulose and hemicelluloses respectively. Efficient utilisation of these sugars is crucial for the cost-effective and sustainable production of chemicals and fuels from lignocellulosic biomass. The industrial workhorse for ethanol production, Saccharomyces cerevisiae, lacks the ability to utilise non-hexose sugars. Genetically modified S. cerevisiae can ferment glucose and xylose, however, a major bottleneck in achieving efficient and cost-effective co-fermentation of mixed sugars is the preferential uptake of glucose that delays the uptake of non-glucose sugars1. Recent studies demonstrated that the partial hydrolysis of cellulose to cellobiose instead of glucose, uptake and intracellular hydrolysis of cellobiose is an option for the alleviation of glucose repression by an engineered S. cerevisiae expressing a cellobiose transporter and an intracellular beta-glucosidase2. Moreover, this enables the co-fermentation of cellobiose and other non-glucose sugars. In this study, we aim to engineer an industrial, xylose-fermenting strain of S. cerevisiae to also ferment cellobiose via a combinatorial genome editing method. Our genome editing approach involves the advanced genome editing tool CRISPR-Cas9 for marker-free multi-copy gene integration in the yeast genome. The cellobiose uptake and intracellular hydrolysis will be optimized by creating a library of transformants with a range of different gene copy numbers, followed by a competitive cultivation scheme to isolate clones with high and fine-tuned levels of gene expression.

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275 Interactions Between S. cerevisiae and Non-Saccharomyces Yeasts During Alcoholic Fermentations

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The use of non-Saccharomyces species in wine fermentations has been increasing in the last decade, mostly due to their positive contribution to the final quality of the wines, and, in some cases, to its ability to reduce ethanol yields. These species are mainly used as mixed inoculum with Saccharomyces cerevisiae, although the interaction between them is not completely understood. The aim of this study was to analyse the interactions between S. cerevisiae and some non-Saccharomyces species of oenological interest (T. delbrueckii, L. thermotolerans, M. pulcherrima) during alcoholic fermentations. Different combinations of strains were used to ferment synthetic must, in sequential and co-inoculation, and at different inoculation ratios. The fermentation kinetics, yeast growth and imposition was followed over time. Our results showed a delay on the mixed fermentations, compared with the single S. cerevisiae inoculation, in most of the cases. Indeed, the imposition of Saccharomyces was impaired in most sequential fermentations. In order to elucidate if the competitive response was due to cell-to-cell contact, we performed a set of fermentations in which the non-Saccharomyces cells were removed from the medium after 48 h, before the inoculation of S. cerevisiae, with and without nutrient supplementation. We also used a dialysis membrane to compartmentalize the mixed cultures. Unexpectedly, the condition in which T. delbrueckii was removed from the medium resulted in stuck fermentations, indicating some inhibitory effect on S. cerevisiae, not dependent on cell-to-cell contact, or nutrient availability.

276 Emerging Links Between TORC1 Signaling And Intracellular Trafficking.

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The target of rapamycin complex 1 (TORC1) kinase is a homeostatic regulator of cell growth that is exquisitely sensitive to the quality and quantity of a diverse array of nutrients. TORC1 functions mainly within the context of intracellular cellular membranes, where it is controlled by and from where it controls intracellular trafficking events by poorly understood mechanisms. Extending the canonical view in which TORC1 functions at the surface of vacuoles, we recently identified a hitherto unknown subpopulation of TORC1 at endosomal membranes, which defines specific signaling endosomes. The activities of endosomal and vacuolar TORC1 are functionally separable from each other and are commissioned with distinct physiological functions. For example, while endosomal TORC1 inhibits macroautophagy by phosphorylating Atg13, vacuolar TORC1 promotes protein translation mainly via Sch9. Using a set of mutants that uncouple endosomal and vacuolar TORC1 activities, we have been able to uncover new TORC1 targets that are more intimately controlled by endosomal TORC1. One of these is Vps27, which is part of the ESCRT-0 heterodimer that acts as gateway complex for protein sorting and degradation through either the multivesicular body (MVB) pathway or microautophagy. In this context, we will not only present our analyses of the molecular details of the respective TORC1-catalysed phosphorylation of Vps27, but also present our latest findings on additional endosomal TORC1 targets that have so far remained elusive. Accordingly, our new data pinpoint the existence of an intimate link between cell growth signaling and intracellular trafficking, two fundamental cellular processes that appear to converge on signaling endosomes.

277 Coordination of Mitochondrial Homeostasis With Cell Size

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Accurate homeostasis of mitochondria is important for cell function. In particular, the amount of mitochondria has to be adjusted according to the size of the cell, which varies about twofold during the cell cycle. Indeed, previous studies have shown that the concentration of mitochondria is maintained roughly constant independent of cell size. However, the molecular mechanisms underlying the coordination of mitochondrial homeostasis with cell growth and size are poorly understood.

To reveal the principles of this coordination, we investigate how the concentration of mitochondrial DNA, mitochondrial proteins and transcripts depends on budding yeast cell size. By controlling the concentration of Whi5, an inhibitor of S phase entry, with a hormone-inducible promoter, we obtain steady state cell populations with mean sizes ranging from 40 to 120 fL.

Using qPCR, we show that the amount of mtDNA increases strongly with cell size. At the same time, the amount of mRNAs encoding mitochondrial proteins needed for mtDNA replication increases with cell size, maintaining roughly constant concentrations. Preliminary flow cytometry results of mCitrine-tagged Abf2 cells suggest that this behavior is also maintained on the protein level. To identify factors essential for mtDNA homeostasis, hemizygous deletions of MIP1 or ABF2 were introduced into diploid strains, which reveal a moderate decrease of mtDNA.

278 Eisosomes in Quiescence

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Quiescence, or G0, the most common but least understood cellular state is crucial for the development and survival of microbial and multicellular organisms. Microbes, including the yeast Saccharomyces cerevisiae, enter this state under nutrient-limiting conditions. Stationary Phase (SP) cultures of S. cerevisiae consist of both Quiescent (Q) and Non-Quiescent (NQ) cells that can be discriminated using density gradient centrifugation (1). Among the proteins identified to be up-regulated in Q cells are proteins found in a specific domain of the plasma membrane, the Membrane Compartment of Can1 (MCC) (2). However, the role of the MCC in G0 remains unknown.

The MCC has been shown to colocalize with eisosomes, subcortical protein scaffolds that correspond to furrow-like invaginations of the plasma membrane. MCCs are upstream regulators of TORC2 in the sensing of lipid homeostasis and membrane stress (3). Among the numerous proteins that partition in MCCs are several nutrient transporters, like Can1, the arginine-specific transporter of yeast. We have recently dissected the conformation-dependent mechanism for the partitioning of Can1 in MCCs (4), and characterised its physiological significance. More specifically, we have shown that MCCs expand in number and size in cells that have reached the SP of growth, protecting Can1 and other transporters from endocytosis and thus allowing the efficient recovery from SP when nutrients become available again (4).

We now examine whether this protection occurs specifically in Q cells. Preliminary evidence suggests that MCCs specifically expand in Q cells, in which several MCC-resident proteins are upregulated. In detail, in Q cells a 4-fold expansion of MCCs is observed, compared to exponentially-growing cells, and MCCs show a network-like architecture rather the patchy distribution in exponential phase. This expansion seems to require Lsp1, a poorly characterized BAR-domain-containing eisosomal protein. The molecular mechanism and pathway via which Lsp1 mediates the expansion of MCCs in SP is currently under investigation. We additionally study the role of MCCs in the survival and recovery of Q cells.

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279 Real-Time Monitoring, Mathematical Modelling and Elucidation of the Morphological Transition of Candida tropicalis from Yeast to Pseudohyphae by Dielectric Spectroscopy.

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Dielectric spectroscopy (DS) has been a key analytical tool in the biotechnology sector for over 25 years; predominantly used to quantify yeast biomass on-line within the brewing industry. The technology exploits the principle that live cells with intact plasma membranes become polarised when subjected to an electric field and that the extent of polarisation, measured as capacitance, is a direct and quantifiable measurement of viable cells within a bioreactor. Aber Instruments Ltd biomass probes are commercially available and utilise dielectric spectroscopy to quantify yeast biomass, however the technology has not been explored to understand the morphological transition of yeast to a hyphal phenotype. Due to the nature of dielectric spectroscopy, AI biomass probes can potentially be used to detect physiological and morphological cellular alterations through analysis of the dielectric spectral range (50 – 20,000 kHz).

Non-albicans Candida species (NACS), such as Candida tropicalis, like many other Candida species exhibit pleomorphism between a yeast and hyphal phenotype that has been associated with the expression of virulence factors linked to pathogenesis. This work presents a method of detecting and correlating the transition of C. tropicalis from a yeast to hyphal phenotype using online monitoring through dielectric spectroscopy, rather than relying on time consuming, offline methods.

Furthermore, we report that regulation of the yeast to hyphal transition in our genome sequenced lab strain, Y4, is reliant and governed by carbohydrate utilisation in planktonic cells. Deconvolution of the dielectric spectrum frequency data has revealed an indicative signature that can be used to reliably model the transition from a yeast to hyphal phenotype. The genetic regulatory mechanisms of this transition will be discussed as will the biomedical and industrial applications of dielectric spectroscopy.

280 Age-Dependent Transitioning of Glutamate Synthase into Large-Scale Assemblies

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Replicative ageing in budding yeast S. cerevisiae is associated with various changes in cellular organelles and metabolism which causes phenotypic heterogeneity within population of cells. However, it is unclear what molecular mechanisms are underlying these age-dependent phenotypic differences. To address this problem and identify what proteins are affected by cellular ageing, we performed a proteome-wide analysis of age-associated protein structural changes using Lip-MS. The results showed that metabolic enzymes, especially those responsible for amino acid metabolism, were highly enriched among the proteins that undergo putative conformational changes. A follow-up microscopic evaluation of the hits revealed that the glutamate synthase Glt1 transitioned from soluble into large scale assemblies during early replicative ageing. The transitioning of various proteins from soluble into various types of assemblies has recently emerged as mechanism to spatially organize biochemical reactions within cells. While most of these assemblies form in response to cellular stress in non-dividing cells, we found that the Glt1 assemblies formed in cycling cells and were asymmetrically inherited by the ageing mother cells during cell division. Further, their appearance correlated with reduced glutamate levels in aged cells, indicating that the transitioning might inactivate the catalytic function of Glt1. We mapped the self-assembly interface using site-directed mutagenesis and identified several mutants that are catalytically active, but are not able to form assemblies. Using these mutants, we hope to unravel how the Glt1 self-assemblies form and what are the consequences of their formation to cellular metabolism and ageing. We propose that these assemblies might function as a metabolic switch and a source of heterogeneity within genetically identical yeast populations.

281 Discovery and Characterization of Xylanolytic Enzymes from Yeast for Industrial Biotechnology Purposes

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Lignocellulosic biomass is one of the main renewable resources that can be used in a future bio-based economy for production of liquid biofuels and biochemicals. For an economically viable process, it is of outmost importance to convert all carbon sources in the lignocellulosic biomass into product. In this respect, xylan is an abundant yet underutilized part of plant biomass that we need to become better at converting into different products.

Much research has been done on xylan-degrading enzymes from bacteria and fungi, while very little is known about which enzymes different yeast species use for the same purpose. Due to the heterogeneity of the polysaccharide, its hydrolysis is performed by a complex set of enzymes among which we find endo-xylanases, xylosidases, arabinofuranosidases and some esterases for the hydrolysis of the different sugars branched to the backbone. A number of yeast species exhibiting xylanolytic activities have previously been isolated from trees, leaves and compostable waste (Ali et al. 2017; Otero et al. 2015; Lara et al. 2014). Among these, we have selected several interesting species with potential to break down xylan and related polymers. The aim of this project is to characterize these yeast species in terms of xylanolytic activity, to understand which enzymes they use and in detail show how the most interesting enzymes function. Our hypothesis is that yeasts produce xylan-degrading enzymes with unique properties (such as new ways to break the various xylan bonds, enzyme structure and stability), which may be useful to industry.

282 Nba1 is Involved in Cell Abscission During Cytokinesis.

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Cytokinesis divides the cytoplasm of a single cell into two distinct cells. In the budding yeast Saccharomyces cerevisiae this last phase of the cell cycle occurs at the bud neck. Here, the assembly and constriction of an actomyosin ring (AMR), the formation of a chitinous primary septum (PS) and the targeted membrane deposition of two glucan- and mannan-rich secondary septa (SS) take place in a precisely executed choreography. Abscission is the final step of the separation of the mother and the daughter cell and is accomplished by the degradation of the PS and parts of the SS at the daughter site.

The Nap1- and bud neck- associated protein Nba1 is recruited to the site of cytokinesis by Gps1. Previous studies showed, that Nba1 prevents a re-polarisation at the old division site during the next cell cycle by locally preventing the activation of the Cdc42-GEF Cdc24 [1]. Our analysis of the genetic interactions between NBA1 and different cytokinesis genes supports an additional role of Nba1 during abscission. Time-resolved protein interaction analysis further shows that Nba1 interacts with the cytokinesis proteins Hof1, Cyk3 and Boi1/2 at the bud neck during cell abscission. An array-based interaction screen revealed new binding partners of Nba1 with potential roles in cytokinesis. SPLIFF analysis again shows that these interactions occur at the bud neck during cell division. We created alleles of NBA1 carrying mutations in the different binding sites to its interaction partners and present our analysis on their effects on bud site selection and cell separation.

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283 A Mechanistically Detailed Model of the Cell Division Cycle in Saccharomyces cerevisiae.

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Understanding how cellular functions emerge from the underlying molecular mechanisms is a key challenge in biology. This will require computational models, whose predictive power is expected to increase with coverage and precision of formulation. Genome-scale models revolutionised the metabolic field and made the first whole-cell model possible. However, the lack of genome-scale models of signalling networks blocks the development of eukaryotic whole-cell models. Here, we present a comprehensive mechanistic model of the molecular network that controls the cell division cycle in Saccharomyces cerevisiae.
This model is mechanistic, as the connections within the model correspond to direct biochemical reactions or dependencies of these reactions on the state of the reactants, down to the level of specific residues and domains. It is genome-scale, as it accounts for all components in the cell division cycle for which we could assign a mechanistic function. We use rxncon, the reaction-contingency language, to neutralise the scalability issues preventing formulation, visualisation and simulation of signalling networks at the genome-scale, and use parameter-free modelling to analyse the cell cycle control network. Interestingly, we find that this network consists of three distinct regulatory modules that control and communicate via three independent replication cycles: DNA replication, SPB duplication and nuclear division, and cell division, and that only a hybrid model including both parts can mechanistically explain cell cycle control. Finally, we benchmark the model on a set of 85 mutants, capturing 62 out of 85 phenotypes. This mechanistic genome-scale model offers

a new perspective on eukaryotic cell cycle control, and opens up for similar models - and eventually whole-cell models - of human cells.

284 Genome-wide Imaging Screen Uncovers Molecular Determinants of Arsenite-induced Protein Aggregation and Toxicity.

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Exposure to toxic metals such as cadmium and arsenic results in widespread misfolding and aggregation of cellular proteins. How these protein aggregates are formed in vivo and the mechanisms by which cells prevent their accumulation during environmental stress is not fully understood. To find components involved in these processes, we used high-content microscopy and identified yeast deletion mutants with either enhanced or reduced protein aggregation levels during arsenite exposure. Analysis of this gene catalogue revealed that mutants with reduced aggregation levels are enriched for functions related to protein biosynthesis and transcription whilst functions related to cellular signalling, metabolism, and protein folding and degradation are overrepresented among mutants with enhanced aggregation levels. Several of the identified genes have functions in signalling and gene regulation, and were previously not linked to protein aggregation. We provide evidence that accurate transcriptional control is crucial for aggregate management and survival during arsenite exposure. On a genome-wide scale, protein aggregation correlated with arsenite sensitivity indicating that many of the factors identified in this study are crucial to withstand environmental stress. Elucidating their molecular roles will increase our understanding of stress-dependent aggregate management and provide insights into the mechanisms of arsenic toxicity and pathogenesis.

285 Nuclear Envelope Budding is an Evolutionary Conserved Phenomenon that Increases During Heat Shock in Budding Yeast

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A fundamental step in the evolution of eukaryotes was the envelopment of genetic material within the nucleus. It is a long-held belief that all transport between the cytoplasm and nucleus occurs via the nuclear pore complex, although several reports suggest transport from the nucleus can also occur via budding of the nuclear membranes (e.g. during Herpes simplex infection). The contents of nuclear budding events (NBEs) has even been identified in Drosophila melanogaster larvae, in which messenger ribonucleoprotein particles m(RNPs) are transported from the nuclear membrane by NBEs. It has also been suggested that NBEs transport damaged proteins in aggregates out of the nucleus. However, it is unknown whether nuclear budding is a conserved process within healthy, fully differentiated eukaryotes as most studies to date were conducted in infected cells or cells of various development stages.

We have used electron microscopy, tomography and immuno-EM of cryoimmobilized cells to investigate NBEs presence, ultrastructure and cargo. We observed and quantified NBEs in five different species, spanning from Trypanosoma brucei to a human cell line. We show that nuclear budding is present in representatives from all branches of the eukaryotic evolutionary tree. Immuno-EM revealed that NBEs are, for the most part, distinct from assembly intermediates or improperly assembled nuclear pore complexes. Furthermore, NBEs occur more frequently in Saccharomyces cerevisiae cells that have been subjected to heat shock. Ubiquitin is detected within NBEs, in agreement with the cargo being either protein aggregates or mRNPs. Current experiments investigate if both cargoes exit the nucleus via budding. Our findings show that nuclear budding is an evolutionary conserved phenomenon by which material is transported across the nuclear envelope, and implicate the process may play a role in protein quality control and translocation of mRNPs across the nuclear envelope.

286 Molecular Evolution of the members of Snq2/Pdr18 subfamily of PDR transporters in Hemiascomycete yeasts

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The Pleiotropic Drug Resistance (PDR) family of transporters is involved in Multidrug Resistance (MDR) in the Hemiascomycetes (1,2). PDR18 gene encodes an ergosterol transport at the plasma membrane, conferring MDR to ethanol, acetic acid, pesticides and metal cations in Saccharomyces cerevisiae (1). PDR18 is a paralog of SNQ2 gene that also confers MDR but to different sets of chemical compounds with little overlapping (3). A previous phylogenetic and neighborhood analysis support the idea that a single duplication event occurring in the common ancestor of the Saccharomyces genus yeasts was at the origin of PDR18 and SNQ2 (3). In this work we describe the analysis of 171 hemiascomycetous genomes, corresponding to 68 species and spanning 7 different taxonomic families plus 4 additional early-divergent species that allowed the identification of 263 members of the PDR Snq2/Pdr18 gene subfamily. All four early-divergent species examined lacked a Snq2/Pdr18 homolog, suggesting that horizontal gene transfer was at the origin of this PDR gene in the late divergent yeasts.

The comparison of the chemical susceptibility profiles of S. cerevisiae snq2Δ and PDR18Δ with those obtained for C. glabrata snq2Δ strain suggests that SNQ2 and PDR18 paralog genes undergone strong functional diversification (3). The aBSREL estimation of dN/dS indicate that episodic positive selection played a determinant role in the functional differentiation of PDR18, suggesting that this gene undergone a process of neofunctionalization. This result was corroborated by the MEME model and MrBayes inference of the amino acid sequence of the corresponding ancestral genes, allowing the identification of 69 codon sites with signs of the past action of episodic positive selection immediately after the formation of the PDR18 gene lineage. The dN/dS rate estimated by aBSREL model did assigned statistical significance to episodic positive selection immediately after the formation of SNQ2 gene lineage. Strong positive selection was active over one gene copy generated at the Whole Genome Duplication (WGD) event, forming a short-lived sub-lineage. Purifying selection was predominant over the other WGD copy, suggesting that the physiological function of the single gene encoded in the protoploid yeasts was preserved in the WGD sub-lineage originating both SNQ2 and PDR18 genes. The majority of the members of the Snq2/Pdr18 subfamily encoded in pathogenic Candida genomes were found comprised in a single gene lineage.

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287 Cellular Control of Mitochondrial DNA Levels During Prolonged Cell-Cycle Arrest in Saccharomyces cerevisiae.

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Mitochondrial DNA (mtDNA) replication is orchestrated by nuclear-encoded proteins but occurs independently of nuclear DNA (nDNA) replication. It has been previously shown that cell-cycle arrest induces excessive accumulation of mtDNA molecules in Saccharomyces cerevisiae cells. On the one hand, mtDNA overreplication decreases yeast viability during prolonged (more than 10 hours) cell cycle arrest in S-phase. On the other hand, yeast cells can survive extremely long cell-cycle arrest in G1-phase induced by destabilization of SCF ubiquitin-protein ligase complex cdc34. Thus, we suggested that there are mechanisms limiting mtDNA replication under prolonged cell cycled arrest. We also suggested that Rho- mitochondrial DNA with large deletions can avoid such control mechanisms. To test these suggestions we studied the accumulation of the wild type (Rho+) and the mutant (Rho-) mtDNAs in yeast cell arrested in different phases of the cell cycle. We used yeast strains with thermosensitive mutations in the genes that induce arrest in different stages under the restrictive temperatures: cdc13-1 (S/G2), cdc15-2 (telophase), Δcdc26 (metaphase), cdc34-2 and cdc53-1 (G1/S). MtDNA/nDNA ratios were estimated using qPCR. Additionally, total DNA content was analyzed by measuring the fluorescence of the DNA-intercalating agent propidium iodide (PI) using flow cytometry. Surprisingly, we did not observe a significant increase in the mtDNA/nDNA ratio in the arrested cells regardless of their mtDNA genotype after 9 h or 24 h of cell cycle arrest in any stage by the qPCR analysis (n=4-6). However, we did observe an increase in the total fluorescence of PI in the arrested cells that indicated an accumulation of DNA molecules different from those that are detected by qPCR. We propose that these fragments can be abortive mtDNA replication products or rDNAs. Together, our data suggest that there are mechanisms limiting excessive mtDNA accumulation during prolonged cell cycle arrest in yeast. Deletion of large fragments in mtDNA does not allow to evade these mechanisms.

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288 Induction of Abnormal Protein Aggregation – A High-Content Imaging-based Screening of FUS Foci Formation in S. cerevisiae.

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The multifunctional DNA/RNA-binding protein FUS has been implicated in the progression of certain forms of neurodegenerative diseases, such as ALS (amyotrophic lateral sclerosis) and FTLD (frontotemporal lobar degeneration). ALS is a progressive and eventually fatal neurodegenerative disorder with characteristics including loss of upper and lower motor neuron functions, partly due to the accumulation of misfolded FUS protein depositions in the cytosol. FUS is predominantly localized in the cell nucleus, where some of its functions include gene regulation and mRNA transport. When mutated, FUS has been shown to locate to the cytosol, forming toxic inclusions in the spinal cord and brain of ALS/FTLD-FUS patients. FUS is thereby lost from the nucleus, most likely resulting in the loss of normal nuclear functions and/or gain of new toxic functions in the cytoplasm.

Saccharomyces cerevisiae contains no FUS homolog, but many cellular pathways coupled to FUS and stress signaling are shared between human and budding yeast cells. When expressed in yeast, full-length FUS assembles into multiple cytoplasmic inclusions due to differences in the nuclear import process of yeast and mammalian cells. Expression of human FUS in yeast will thereby result in foci formation resembling the inclusions formed during ALS pathogenesis. This behavior was applied in a yeast high-content imaging-based screen to investigate the impact of inducible FUS-RFP aggregation on proteins in the yeast GFP library. This approach enabled us to visualize the effects of FUS aggregation on over 4500 different GFP-tagged yeast proteins.

From the screen, we identified proteins that display induced aggregation due to FUS foci formation. Many of these protein aggregates also show a significant overlapping fluorescent signal with the FUS foci, suggesting a possible physical interaction between the aggregates. The initial results from the screen indicate that many of the proteins that form aggregates, upon FUS foci formation, are involved in mRNA binding and processing, as well as being components of the stress signaling pathway. By further studying the interaction between our screen hits and FUS, we wish to uncover and decode the cytotoxic effects of FUS aggregates seen in the progression of neurodegenerative disease.

289 Mapping Genetic Influences on Ubiquitin-Proteasome System Activity

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The ubiquitin-proteasome system (UPS) is the cell's primary pathway for targeted protein degradation. Variation in UPS activity occurs in response to a host of changes in cellular environment, but to what extent UPS activity is shaped by heritable genetic variation is largely unknown. Specifically, we do not know which DNA sequence variants in a population affect UPS activity and the mechanisms by which they exert their effects. This limits our understanding of how genetic variation contributes to UPS function and phenotypic variation in cellular and organismal traits influenced by the UPS, including the many human diseases marked by aberrant UPS activity.

We developed a new method to characterize the genetic architecture of UPS activity in the yeast Saccharomyces cerevisiae. Our approach combines novel fluorescent reporters of UPS activity with statistically powerful bulk segregant genetic mapping to systematically probe the genome for sequence variation influencing UPS activity. We built and characterized dual-color fluorescent reporters that assay the activity of multiple UPS pathways, including ubiquitin-independent UPS protein degradation and the Arg/N-end and Ac/N-end pathways. We mated genetically divergent strains harboring these reporters and sporulated the resultant hybrid to create populations of millions of genetically variable cells. From these populations, we selected pools of cells from the high and low tails of the UPS activity distribution and performed pooled whole-genome sequencing to identify genomic regions with sequence variants affecting UPS activity.

Our results show that UPS activity is a genetically complex trait. We identified a median of 10 regions influencing UPS activity per reporter. Several regions were common to all reporters and converged on promising candidate causal genes. For example, one region centered on the RPN4 gene, which encodes a transcription factor for proteasome genes. Other regions contained genes with no known link to UPS function. In addition, we identified several regions that were unique to individual UPS reporters, including a region on chromosome IV that specifically influences the Arg/N-end pathway. We are currently fine-mapping candidate causal genes and nucleotides in these regions to determine the molecular mechanisms by which sequence variants shape UPS activity. Our results provide the first systematic demonstration of how genetic variation creates heritable differences in UPS activity.

290 Nicotinamide Nucleotide Damage and Repair in Yeast.

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Sequence database analyses show that, even in a well-characterized organism like Saccharomyces cerevisiae, up to 2000 proteins have still incomplete functional annotations. Around 600 of these yeast proteins are predicted to carry a catalytic function. Recent findings suggest that a possibly significant fraction of these 'unknown' enzymes are involved in a process called metabolite repair. Abnormal metabolites are constantly generated inside the cell by unwanted chemical reactions or by enzymatic side reactions; they are useless at best and toxic at worst. Metabolite repair enzymes clear the metabolite pool of these non-canonical metabolites. In 2011, we identified the gene encoding one important metabolite repair enzyme in yeast, namely NAD(P)HX dehydratase. This led to the discovery of an additional enzyme, NAD(P)HX epimerase, that contributes to the removal of potentially toxic NADH and NADPH derivatives. Both enzymes turned out to be widely conserved across species. NADHX, a hydrated form of NADH, can be produced in a side reaction catalyzed by GAPDH, but NADHX and NADPHX can also be formed spontaneously from NADH and NAPDH under conditions of low pH or increased temperature. In-depth phenotyping of an NAD(P)HX repair-deficient yeast mutant allowed us to demonstrate NADHX accumulation accompanied by NAD depletion in post-diauxic growth stages in this strain. Importantly, decreased serine levels pointed us towards a potent inhibitory effect exerted by NADHX at the level of the first step of the main serine synthesis pathway, catalyzed by 3-phosphoglycerate dehydrogenase. These investigations led to another discovery, namely that this latter enzyme oxidizes 3-phosphoglycerate via a transhydrogenase mechanism involving the formation of 2-hydroxyglutarate and apparently rendering serine synthesis less dependent, in S. cerevisiae, on the redox state of the cell. The physiological importance of the NADHX repair system is now strikingly illustrated through a recently identified fatal neurometabolic condition that develops in young children deficient in this repair system. The molecular mechanisms involved in this disease and potential therapeutic strategies are currently being investigated.

291 Hsp90 Immunophilin Homolog Cpr7 Is Required For [URE3] Prion Propagation In Yeast

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Chaperone machinery consists of Hsp70 and Hsp90 family proteins whose activity are regulated by their co-chaperones. The role of Hsp70 and its co-chaperones are well established for yeast prion propagation. Though Hsp90 is highly conserved in eukaryotes and play critical roles in cellular processes like client maturation, protein degradation, apoptosis and cellular signaling but its role in modulation of amyloids like aggregates is not clear. The Hsp90 reaction cycles is regulated by dynamic association with various co-chaperones broadly divided into TPR or non-TPR containing proteins such as Sti1, Cpr7 and Aha1 respectively. Here we have examined the role of Hsp90 chaperone machinery on [URE3] stability. In our study we have expressed Hsp82 variants as a sole source of Hsp90 and we shown deletion of MEEVD pentapeptide motif of Hsp90 results in loss of [URE3] stability. Hsp90-MEEVD motif is required for interaction with TPR domain containing co-chaperones of Hsp90. We knocked out Hsp90 co-chaperones in [URE3] and observed that deletion of Cpr7 encoding gene results in loss of [URE3] prion. Chimeric domain studies suggest that TPR domain of Cpr7 is required for [URE3] prion propagation and helps in [URE3] propagation by influencing Ure2 fibril formation. In our study we have found that Cpr7 is required for [URE3] prion propagation. Our future direction is to explore Cpr7 and its human counterpart as a therapeutic target to combat amyloid based diseases.

292 2-Hydroxyglutarate as a Regulator of Chromatin and Gene Expression

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2-hydroxyglutarate (2HG) is an oncometabolite, identified at abnormal concentrations in neurometabolic diseases and several types of cancer. However, the impact of 2HG on disease initiation and development is still unclear. In cancer, the accumulation of 2HG is caused by gain of function mutations in an isocitrate dehydrogenase (IDH) enzyme and can inhibit several alpha-ketoglutarate-dependent enzymes by acting as a competitor to alpha-ketoglutarate. TET and JmjC-domain containing enzymes responsible for DNA and histone demethylation, respectively, can notably be inhibited by high levels of 2HG. However, the direct consequences of 2HG accumulation on DNA stability and gene expression remain elusive. By using Saccharomyces cerevisiae as a model organism, we investigate the effects of 2HG at the epigenome-wide level. Yeast is devoid of DNA methylation. Thus, using yeast as a model organism allows us to focus on the impact of 2HG on the histone modifications related to active transcription. As a first step, genetically modified yeast strains accumulating high and low levels of 2HG have been compared at the level of their metabolic and gene expression changes. The transcriptomic changes upon the accumulation of 2HG depend on the genetic background of the yeast and a pathway enrichment analysis highlighted changes in amino acid metabolism in response to higher levels of 2HG. By performing chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) we are investigating the alterations in histone methylation caused by the elevated levels of 2HG and their association with the gene expression changes. In particular, we focus on H3K4me3 and H3K36me3, the two histone modifications associated with transcription start sites and active transcription, respectively. Overall, this study aims to provide better understanding on the gene expression and metabolic pathways affected by the 2HG concentration.

293 Characterization of a Novel Member of the Yeast Polarisome With a Role in Actin Organization.

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Polarity establishment in the budding yeast (Saccharomyces cerevisiae) is a highly regulated process. The polarisome comprises a network of proteins that organizes polar growth in yeast and filamentous fungi. Spa2, the yeast formin Bni1 and the actin-nucleation-promoting factor Bud6 are subunits of the polarisome that together catalyze the formation of actin filaments below the tip of yeast cells. We identified YFR016c (Aip5) as interaction partner of Bud6 and the polarisome scaffold Spa2. Yeast cells lacking Aip5 display a reduced number of actin cables. Aip5 binds with its N-terminal region to Spa2 and with its C-terminal region to Bud6. Both interactions collaborate to localize Aip5 at bud tip and neck, and are required to stimulate the formation of actin cables. Supported by genetic analysis and fluorescence microscopic experiments we postulate that Aip5 assists in the polarisome mediated assembly of actin filaments.

294 A High-Content Microscopy-Based Screen for Factors Involved in Aggregate Deposition at Mitochondria.

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Loss of protein homeostasis is a hallmark of aging and the formation of protein aggregates is characteristic for diseases such as neurodegenerative diseases, diabetes and cancer. Since aggregated proteins can disrupt cellular function, they are often sequestered into inclusions and deposited at specific sites in the cell by the spatial quality control machinery. It has recently been shown that inclusions can be deposited at or near mitochondria using the disaggregase, Hsp104, as a marker and we have confirmed this co-localization with three different misfolding reporter proteins. The significance of this specific co-localization with regard to quality control is unclear. We therefore performed a genome-wide high-content microscopy-based screen to find factors necessary for deposition of aggregates at mitochondria. Two main processes were identified: protein turnover and mRNA processing. While protein turnover was an anticipated finding, the involvement of mRNA processing in aggregate sequestration at mitochondria was surprising. Candidates from both processes were further investigated.

295 Molecular Mechanisms of Clearance of Arsenite-induced Protein Aggregates in Saccharomyces cerevisiae.

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The poisonous metalloid arsenic manifests its toxicity in vivo by causing widespread misfolding and aggregation of nascent proteins. To protect themselves against the harmful accumulation of protein aggregates, cells use an array of protein quality-control mechanisms. Molecular chaperones assist the folding of proteins into their functional conformation or help misfolded proteins to regain their native structures. Moreover, molecular chaperones are integral parts of protein degradation systems, such as the proteasome, the autophagy pathway, and the lysosome/vacuole, that eliminate misfolded and aggregated protein conformers. In this work, we addressed the mechanisms of aggregate clearance using the yeast Saccharomyces cerevisiae. Role of proteasome, authophagy, and vacuolar protein degradation pathways as well as chaperone-mediated disaggregation and refolding was systematically probed.

296 The Role of Cdc42p Adaptors in Regulating the Filamentous Growth MAP Kinase Pathway.

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Rho GTPases regulate cell polarity and signal transduction pathways to control morphogenetic responses in different settings (1). In yeast, the Rho GTPase Cdc42p regulates cell polarity and mitogen-activated protein kinase (MAPK) pathways [mating, filamentous growth or fMAPK, and HOG (2)]. Although much is known about how Cdc42p regulates cell polarity and mating, how Cdc42p is activated in the fMAPK pathway is not clear. This question is relevant because filamentous growth is a developmental fungal response to nutrient limitation that occurs in many fungal species, including pathogens (3-5). Moreover, although the signaling glycoprotein (Msb2) that regulates the fMAPK pathway has been characterized (6-7), how Msb2p connects to Cdc42p is not clear. To begin to address this question, Cdc42p-dependent MAPK pathways were compared in the filamentous (∑1278b) strain background. Each MAPK pathway showed a unique activation profile, with the fMAPK pathway exhibiting slow activation kinetics compared to the mating and HOG pathways. A previously characterized version of Cdc42p, Cdc42pE100A (8), that is specifically defective for fMAPK pathway signaling, was also defective for interaction with Bem4p, the pathway-specific adaptor for the fMAPK pathway (9). Corresponding residues in Bem4p were identified that were required for interaction with Cdc42p and fMAPK pathway signaling. The polarity adaptor Bem1p also regulated the fMAPK pathway. In the fMAPK pathway, Bem1p recruited the p21-activated kinase (PAK) Ste20p to the plasma membrane, cycled between an open and closed conformation, and interacted with the GEF Cdc24p. Bem1p also regulated effector pathways in different ways, behaving as a multi-functional adaptor in some pathways and as a passive scaffold in others. Genetic suppression tests showed that Bem4p and Bem1p, together with Rsr1p – the GTPase that controls bud-site-selection and also regulates the fMAPK pathway (10) - regulated the fMAPK pathway in an ordered sequence. Collectively, the study demonstrates the unique and sequential roles that Rho GTPase adaptors and regulators play in the control of MAPK signaling.

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297 Superoxide Induces Adaptive Editing of Mitochondrial DNA

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Mitochondria are continuously exposed to reactive oxygen species (ROS) generated as a byproduct from respiration. Damage from oxidative stress may lead to impaired mitochondrial function, a common feature of cancer and aging cells. We sought to understand adaptation to elevated ROS levels in yeast by adapting 1152 populations to elevated superoxide exposure and following growth phenotypes for approximately 250 generations. Adaptation to superoxide was exceedingly fast leading to almost complete superoxide tolerance after only 10 generations. We show that superoxide adaptation is driven by a regulated rapid degradation of mtDNA leading to loss of key mitochondrial respiratory electron transport genes. MtDNA and respiratory capacity could be fully restored by removing stress from early adapted populations. In contrast, chronic superoxide exposure leads to complete mtDNA erosion resulting in permanent irreversible adaptation to superoxide and permanently lost respiratory capacity. These results imply a regulated adaptive mechanism of degrading mtDNA as a response to superoxide stress through which chronic exposure leads to mitochondrial distress.

298 Peroxiredoxin promotes longevity and H_2O_2-resistance in yeast through redox-modulation of protein kinase A-dependent nutrient signalling

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Peroxiredoxins are modulators of aging in yeast and multicellular organisms (1,2). We have previously identified key roles for the peroxiredoxin Tsa1 in mechanisms by which caloric restriction boosts yeast H_2O_2 stress resistance and slows down replicative aging (3,4). The mechanisms by which peroxiredoxins stimulate H_2O_2 resistance and slow down aging are, however, still largely unclear. In our recent studies we can show that the yeast peroxiredoxin Tsa1 boosts H2O2 resistance and prevents aging without affecting cellular H_2O_2 levels. Specifically, we pin-point a role of Tsa1 as an H_2O_2-receptor inhibiting nutrient signaling via protein kinase A (PKA) that governs both H_2O_2 resistance and longevity. Tsa1 controls PKA activity in a manner independent of the second messenger cAMP at the level of a conserved cysteine in the catalytic subunits. Stimulating Tpk1 redox-modification at this specific cysteine residue inhibits PKA activity and boosts cellular H_2O_2 resistance in part through dephosphorylating a conserved threonine in the kinase activation loop. Strikingly, preventing the phosphorylation of this threonine counteracts the H_2O_2 sensitivity of cells lacking Tsa1. These results will be discussed in the context of an integrative model of aging where nutrient signalling pathways constitute hubs integrating information from multiple aging-related conduits and where the activity of a protein kinase can be fine-tuned by redox-modification through peroxiredoxins.

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299 Multiple Functions Of The Nuclear Pore Complex In The Replication Cycle Of The Ty1 Retrotransposon.

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Transposable elements (TEs) are major components of genomes and play a fundamental role in shaping genomes throughout evolution. However, TE integrations can be detrimental for genetic integrity in the short-term. Retrotransposons are related to retroviruses and tend to integrate at given positions in the genome. Deciphering how retrotransposons interact with cellular factors to replicate and integrate at preferred sites is thereby fundamental to understand their contribution to genome dynamics. In S. cerevisiae, the Ty1 LTR-retrotransposon preferentially integrates into a 1-kb window upstream of RNA polymerase III (Pol III)-transcribed genes, i.e. tDNAs. We showed previously that the Pol III subunit AC40 is a predominant determinant of Ty1 integration site selection. Yet the spatio-temporal regulation of this process remains to be characterized.

Many links have been noticed between the replication cycle of retroelements, which takes place both in the nucleus and the cytoplasm, and Nuclear Pore Complexes (NPCs), the unique connections between these compartments. In particular, NPCs were shown to contribute to optimal replication and integration site selection but the underlying molecular mechanisms are still undefined. To disentangle the multiple functions of NPCs in Ty1 retrotransposon replication, we combined genetic and genomic approaches together with classical tools to study Ty1 biology in relevant S. cerevisiae NPC mutants. This genetic dissection of NPC functions revealed that Nup84C, a major structural NPC scaffold, is a pivotal component at distinct stages of Ty1 replication cycle, from transcription to integration. In particular, by performing a high-throughput sequencing of hundreds of thousands of de novo Ty1 insertion sites in distinct NPC mutants, we showed that Nup84C is a major determinant in the preference of Ty1 integration upstream of tDNAs. Strikingly, tDNA transcription can occur at the NPC, raising the possibility that Nup84C-dependent tDNA anchoring could favor local Ty1 integration.

Our data thereby suggest a model in which NPC-dependent genome organization orchestrates Ty1 integration site selection at a step upstream of AC40 tethering factor. This study provides innovative insights into a novel function of NPCs in the regulation of genome expression and integrity through complex interactions with retrotransposons.

300 Identification of Modifications Procuring Growth on D-xylose in Recombinant Saccharomyces cerevisiae Strains Carrying the Weimberg Pathway

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In order to compete with fossil fuel-dependent bulk chemicals production, biorefinery processes must utilize all major sugars found in the raw material. In lignocellulosic biomass, up to a third of the sugars consists of xylose, a pentose sugar that the industrial workhorse S. cerevisiae cannot naturally use. The most prevalent xylose-assimilating pathways in recombinant S. cerevisiae, i.e. the xylose isomerase and the xylose reductase/xylitol dehydrogenase pathways, channel the carbon flux through the pentose phosphate pathway and further into glycolysis. In these yeast strains, co-consumption with the most common lignocellulosic sugar, glucose, is challenging since xylose enters yeast metabolism in the upper part of glycolysis, competing with the glucose flux.

Several bacterial and archaeal species have been found to assimilate xylose via oxidative and non-phosphorylative routes, such as the Weimberg or the Dahms pathways, that end with the products α-ketoglutarate (αKG) or pyruvate and glycolaldehyde, respectively. If introduced in S. cerevisiae, the inferred bypass of glycolysis would enable production of value-added specialty chemicals without carbon loss or inhibition from hexose catabolism.

Previous attempts to establish the Weimberg pathway from the bacterium C. crescentus in S. cerevisiae have resulted in accumulation of the growth restricting intermediate xylonate, supposedly due to limitations in the lower pathway. In the present study, deregulation of iron metabolism, proteomics and adaptive laboratory evolution were used to pinpoint rate-limiting steps in the Weimberg pathway expressed in the yeast. It was thereafter possible to engineer S. cerevisiae to enable growth on xylose. To our knowledge this is the first report of a functional complete Weimberg pathway expressed in fungi. After optimization this pathway could be used for production of carboxylic acids and diols from xylose, via αKG and the Weimberg route.

301 Galactose in the N-Glycans of the yeast Yarrowia lipolytica

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The yeast Yarrowia lipolytica has an excellent protein secretion capacity and is non-pathogenic, what makes it an attractive production platform for therapeutic glycoproteins[1]. Yeasts however, modify glycoproteins with non-human high mannose-type N-glycans that reduce the protein half-life in vivo and can be immunogenic to humans. Our study revealed that N-glycans in Y. lipolytica contains over 22% of galactose in their structures, what makes this yeast even better candidate than previously thought for production of humanized glycoproteins.

The aim of this work was to identify and characterize genes encoding enzymes involved in the N-glycosylation pathway in Y. lipolytica. Five genes, namely GAL10E (YALI0E26829g) encoding UDP-galactose epimerase, two encoding potential galactosyltransferases (YALI0E01034g, YALI0E12199g) and two potential UDP-galactose transporters (YALI0E22957g, YALI0F23089g) were investigated. The analyses showed, that GAL10E, an enzyme from the Leloir pathway of galactose metabolism, is necessary to provide galactose for N-glycosylated proteins. Mutants with overexpression of galactosyltransferases and/or UDP-galactose transporters varied in the monosaccharide content of N-glycans. Overexpression of YALI0E12199g, a potential galactosyltransferase gene resulted in a phenotype unable to grow in media containing hygromycin B. These observation confirms previous findings, that Y. lipolytica mutants affected in Golgi N-glycosylation are more sensitive to this antibiotic[2].

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302 Identification of extracellular residues that anchor the interaction between the pore-forming subunit Cch1 and the essential regulatory subunit Mid1 of a yeast Ca2+ channel

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Saccharomyces cerevisiae has a Ca2+ channel related to mammalian voltage-gated Ca2+ channels (VGCCs) and Na+ leak channels non-selective (NALCNs). This channel is composed of two essential subunits Cch1 and Mid1. Cch1 is a homolog of the pore-forming α1 subunit of VGCCs and NALCNs, while Mid1 has no homologous proteins in animal cells. But some features of Mid1 resemble those of animal α2/δ subunits, such as a C-terminal Cys-rich region.

The role of the Cch1/Mid1 channel are elucidated considerably. This channel is required for cellular responses to mating pheromone, ER stress, alkaline stress and cold stress. However, activation mechanisms underlying the responses remain unknown. To unravel the mechanisms, it is important to understand the molecular interaction between the two subunits.

To study the interaction, we first focused on nine Cys residues in the putative extracellular regions of Cch1 and found that eight of the nine were conserved. Alanine scanning mutagenesis revealed that the eight Cys residues were required for Ca2+ influx. Co-immunoprecipitation experiments revealed that two (Cys-1369 and Cys-1379) of the eight were necessary for the interaction of Cch1 with Mid1. The two Cys residues were found to be present in the loop between S5 segment and the pore of domain III (III-S5P).

To further identify amino acid residues important for Cch1-Mid1 interaction in domain III, we performed PCR-based random mutagenesis with manganese on the CCH1 DNA fragment that corresponds to the region around the III-S5P and identified four residues that were involved in the interaction of Cch1 with Mid1, illuminating the importance of the extracellular loop between S5 segment and the pore in domain III in molecular interaction between Cch1 and Mid1.

To identify Mid1's residues that participate in interaction with Cch1, we focused on the C-terminal Cys-rich region that contains conserved 12 Cys residues. Alanine scanning mutagenesis revealed that three of the 12 Cys residues were essential for the Ca2+ influx-mediating activity of Mid1. Co-immunoprecipitation experiments revealed that one of the three, Cys-498, was found to be required for the interaction of Mid1 with Cch1.

Further study suggested that the Cch1-Mid1 interaction did not depend on disulfide bonding. Thus we suggest that non-covalent bonding between several residues in the Cch1 III-S5P loop and the Mid1 Cys-498 are involved in interaction between the two subunits.

303 ILF and VRED Pathways Prevent Vacuole/Lysosome Rupture by Oxidative Stress.

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A consequence of cellular aging is the accumulation of damaged biomaterials and the progressive reduction in organelle functionality. In yeast, replicative aging is accompanied by an increase in the production of reactive oxygen species (ROS). Yeast vacuoles, lysosomes in higher eukaryotes, are important acidic organelles that combine enzymatic conversion and recycling of the cellular waste with nutrient sensing, storage, signaling, and mobilization. The lysosome also stores metals such as iron, which in the presence of luminal hydrogen peroxide and through Fenton reaction will produce ROS. Elevated ROS can then promote lysosome membrane permeability (LMP), resulting in leakage of luminal hydrolase into the cytoplasm and trigger lysosomal cell death (LCD). Here we have used the yeast S. cerevisiae vacuole as a model to study the mechanisms in how cells prevent lysosome rupture and cell death under oxidative stress. We hypothesize that the cell will down-regulate two membrane proteins that are crucial in regulating luminal ROS: the vacuole iron transporter Fth1 and the iron/copper oxidase Fet5. Our predictions are that the down-regulation is mediated by at least two selective vacuole degradation pathways: the ESCRT-dependent VRED (Vacuole protein REcycling and Degradation) and the ESCRT-independent ILF (IntraLumenal Fragment) pathways. In doing so, a decrease in luminal ROS production would limit the damage to membrane proteins and lipids and prevent cell death by LCD. In vivo treatment of S. cerevisiae cells with 1M H2O2 induces vacuole membrane permeability, resulting in CMAC dye leakage and cell death is observed. Deletion of Fet5 or Fth1 confers resistance to cell death. Mild treatment of cells with H2O2 seems to stimulate homotypic fusion and the formation of vacuole membrane localized vesicles. Furthermore, we observed that Fth1-GFP is sorted into the ILF and VRED pathways for degradation. Deletion of the ESCRT-1 complex component Vps23 does not block Fet5-GFP of being sorted into the VRED pathway but prevents delivery to the vacuole lumen for degradation. We further show that cells lacking genes supposedly required for the VRED pathway are sensitized to H2O2. These mechanisms could protect cells from lysosomal cell death, by limiting lumenal reactive iron levels to prevent ROS generation and preventing aggregation of oxidized proteins to limit vacuole lysosome membrane permeability.

304 Phenomics, transcriptomics and metabolomics for identifying concentration-dependent chemical interactions and understanding the mechanistic basis of the mixture toxicity.

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The prevalence of mixtures of synthetic and natural chemicals in the environment is a growing concern for public health and environmental effects. Currently, most chemical legislations are based on the risk assessments carried out on individual substances and theoretical estimates of combination effect. However, exposure to multi-component mixtures may stimulate unpredicted overall toxic responses due to interactions, where interactions were scored as deviations from the independent action model. In our project, we investigated the frequency and magnitude of interactions in mixtures of five compounds - NaCl, HgCl2, paraquat, rapamycin, clotrimazole - with relatively known specific mode of action. Growth effects by all-combination pair-wise mixtures spanning a wide concentration range were investigated by employing high-resolution yeast phenomics. The baker's/brewer's yeast Saccharomyces cerevisiae and the marine yeast Debaryomyces hansenii are used in this study to identify evolutionary conserved mixture effects, with the aim to identify generic responses of relevance to a vast array of organisms. Our results clearly show that both synergistic and antagonistic relationships exist among the tested chemicals and some of these relationships are concentration-dependent. Evolutionary conserved interactions on the level of rate of growth were found for salt and rapamycin (synergy) as well as for salt and paraquat (antagonism). The mechanistic basis of the chemical interactions identified in our study was investigated by transcriptomics and metabolomics. As one example, we observed that several genes with symporter activity and with cation transmembrane transporter activity is downregulated in salt plus paraquat mixtures, while the expression of genes that are related to cofactor-dependent metabolic pathways is stimulated. We believe that the repression of symporter and ion transmembrane transport activity reduces paraquat entry to the yeast cells and thereby reduces its toxic response when combined with salt. On the other hand, upregulation of several of the genes (such as PGI1, PFK1, FBA1, and CDC19) related to cofactor-dependent metabolic pathways boost yeast fermentative activity. Since paraquat induces the production of reactive oxygen species (ROS) via respiration, a shift from aerobic respiration to anaerobic fermentation can reduce formation of ROS, thus reduces oxidative stress by paraquat.

305 The yeast AP-1 factor Yap8 couples arsenic-sensing to transcriptional regulation of target genes

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Arsenic is a toxic metalloid present in nature and all organisms have developed different mechanisms to evade its toxicity. The Saccharomyces cerevisiae AP-1-like protein Yap8p is a key transcription factor that confers arsenic tolerance by stimulating enhanced transcription of the arsenic-specific detoxification genes ACR2 and ACR3. Yap8 regulates transcription by binding as dimers to specific DNA motifs (YREs, Yap response element). Arsenite binds directly to the three conserved cysteine residues of Yap8p promoting its transcriptional activity. Preliminary data indicates that Yap8 couples arsenic-binding with chromatin remodelling to induce the gene expression. Our data uncover new insights into the molecular mechanism involved in the transcription activation of these genes. The subtelomeric localization of the ACR genes affects their transcription. Changes in the histone acetylation levels and the RNA pol II recruitment require the conserved cysteines of Yap8. Moreover, we have identified multiple Yap8 interacting proteins involved in the chromatin remodeling. These results contribute in the knowledge advance of one of the largest families of transcription factors in eukaryotic cells

306 Fine-tuning the stress response of Saccharomyces cerevisiae using CRISPR interference technology.

Vaskar Mukherjee, Luca Torello Pianale and Yvonne Nygård

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Efficient biochemical conversion of renewable carbon sources is crucial for the transition into an entirely renewable energy system and a resource-efficient society. However, the substitution of fossil based chemicals with renewable biochemicals requires the production to be significantly more efficient and price competitive. Remediation of several technical bottlenecks is needed before this can be accomplished. Production of second-generation biochemicals (made from lignocellulosic biomass) is challenging due to presence of inhibitors in lignocellulosic hydrolysates. Weak acids, furans and phenolic compounds that are formed or released during hydrolysis of biomass are toxic for the producing cells and leads to suboptimal yield and productivity obtained during fermentation. In this project, we are trying to fine tune the expression of stress related genes to boost the stress tolerance in Saccharomyces cerevisiae using the CRISPR interference (CRISPRi) technology. CRISPRi is a genetic perturbation technique that allows sequence-specific repression or activation of gene expression, achieved by a catalytically inactive Cas9 protein fused to a repressor or activator, which can be targeted to any genetic loci using an sgRNA. Using a high-throughput yeast transformation method developed in our laboratory, we are generating a CRISPRi strain library. Each strain in this library has altered regulation for at-least one stress related gene. Next, high-throughput phenotypic evaluation of this library is performed by growing the strains under the exposure of inhibitors relevant to lignocellulosic hydrolysates. Here, we will demonstrate our primary CRISPRi library data. Further, we will explain the high-throughput methodologies for generating the CRISPRi mutants and to study their hydrolysate tolerance, adaptation and ethanol production capacity at microscale. In future, we will perform transcriptomics analysis of the most tolerant mutants to link superior phenotypes to the transcriptomic landscape. Subsequently, this novel information will be used as a resource to accelerate the design-build-test-learn cycle used for developing industrial yeast strains for efficient conversion of lignocellulosic hydrolysate.

307 Nucleolin Rescues TDP-43 Cytotoxicity In A Yeast Model Of ALS Disorder.

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Amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder characterized by the selective loss of motor neurons, is almost invariably associated to the cytosolic mislocalization and aggregation of the nuclear TDP-43 protein. Notably, TDP-43 aggregates are also a histopathologic hallmark of other ALS-related disorders, as fronto-temporal dementia (FTD).

Within the cell, TDP-43 plays multiple roles in transcriptional and post-transcriptional regulation, nucleocytoplasmic trafficking, and stress granules formation. Consistently, TDP-43-dependent impairments of such fundamental processes become extremely damaging to the cells. Most of TDP-43 ALS-linked mutations promote its cytoplasmic aggregation and cytotoxicity, actually mediated by the disordered C-terminal region, where pathogenic mutations have been mainly mapped. Emerging interest is now focusing to the ability of specific proteins to modulate the negative effects of TDP-43 in the cell, possibly indicating novel therapeutic targets. However, the molecular mechanisms driving these complex phenotypes are far to be fully understood.

Here we show the functional characterization of a novel genetic suppressor of TDP-43 damage, the nucleolin protein (NCL), which can almost completely rescue the toxic effects caused by both wt and mutant TDP-43 to yeast cells. Noteworthy, NCL is mainly involved in nucleolus formation, transcriptional regulation (especially of rRNA genes), ribosome biogenesis, and nucleocytoplasmic trafficking.

Data will be presented from multiple assays (i.e., genetic, biochemical and functional) performed in our yeast ALS-model strains, aimed to unveil the molecular mechanisms ruling the specific relationship between TDP-43 and NCL. Collectively, results in yeast and mammalian cells, also indicating some beneficial effects of NCL overexpression for TDP-43-damaged cells, will contribute to discuss the functional implications of our observations for ALS-related disorders.

308 Representing Transcriptional Heterogeneity and Inter-Strain Variation at the Saccharomyces Genome Database.

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One of missions of the Saccharomyces Genome Database is to catalog genome information about Saccharomyces cerevisiae that includes variations in DNA sequence and RNA expression. To present a high resolution view of the transcriptional dynamics in S. cerevisiae, SGD utilized data generated by collaborators (Pelechano et al., 2013) through Transcript IsoForm Sequencing (TIF-seq) technology. This technique captures the heterogeneity of RNA transcription in S. cerevisiae, which can vary widely across experimental perturbations, within different environments, and between cells. The YeastMine data warehouse provides downloadable tables mapping these isoforms to the locations of transcripts identified in a curated group of other genomic datasets. Transcript abundance and outer boundaries can be visualized in the JBrowse genome navigator, and annotation files of all transcripts that fully encompass annotated genes are available from SGD through Amazon Web Services (AWS). The AWS cloud server also hosts variant genotyping files for 25 S. cerevisiae strains (Song et al., 2015) assembled by the Automated Genome Analysis PipelinE (AGAPE), and these single nucleotide polymorphisms and small insertions/deletions are also shown in JBrowse. SGD's Variant Viewer presents the molecular consequence of variants within annotated genes and infers evolutionary relatedness across SGD's 12 reference strains. With these resources in place, we seek to incorporate more transcriptome and strain variant data in concert with improving technical capabilities and a growing collection of datasets.

309 Variation in Processing of Misfolding Proteins.

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Cells have a protein quality control system which can either refold, degrade or sequester potentially harmful misfolded proteins. Misfolded proteins that are not efficiently refolded or degraded are likely to be sequestered into collections of protein aggregates, often called inclusion bodies. These inclusions are hallmarks of aged cells and of several diseases including Alzheimer's disease and Parkinson's disease. While most cellular proteins can misfold, only a subset can be induced to form visible aggregates in the cell and very few misfolding proteins have been linked to disease. This suggests significant differences in the way individual misfolded proteins are handled by the protein quality control machinery but it is unclear what properties of misfolding proteins influence these differences. To address this, we compared processing of three different temperature-sensitive model misfolding proteins, guk1-7, gus1-3, and pro3-1 and found significant differences in aggregation properties and rates of clearance from inclusion bodies. Strikingly, two different proteins localizing to the same site could be cleared at different rates. Our study shows that deposition sites of the spatial quality control machinery may have a more complex organization than is currently appreciated.

310 Loss of ATG1 and YDR131C Together Results in Flocculation Behavior in S. cerevisiae.

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Autophagy is a catabolic biological process, employed by cell during stress conditions such as, starvation, collapsing of cell organelle and infection by pathogens, which helps the cells to survive. The ATG1 gene in S. cerevisae, encodes an Atg1 protein product with serine/threonine kinase activity which requires vesicle formation in autophagy. During autophagy, Atg1p forms complex with Atg13p and Atg17p. The Atg1p also involved in cytoplasm-to-vacuole targeting (CVT) pathway. The cell cycle progression from G2/M to G1 under nitrogen starvation requires the Atg1. The null mutant of ATG1 shown to be viable, however showed the autophagy and cell cycle progression defects. The YDR131C gene in S. cerevisae, encodes for F-box motif containing protein, which constitutes the subunit of SCF-E3 ubiquitin ligase complex. The null mutant of YDR131C shown to be viable and function of its protein product in biological processes has not been yet reported. Here we have investigated the genetic interaction among both the genes, ATG1 and YDR131C (named as NKB31). We report that, the loss of both the genes together (atg1Δnkb31Δ) results in reduced growth and flocculation behavior when grown in YPD medium, however the atg1Δ, nkb31Δ and WT cells showed no such behavior in the BY4741 genetic background. The flock formation behavior by atg1Δnkb31Δ cells was reduced when grown in the presence of EDTA. The atg1Δnkb31Δ cells also showed the sensitivity to acetic acid, ethanol and glycerol in comparison to atg1Δ, nkb31Δ and WT. In conclusion, we suggest that, ATG1 and NKB31 works in parallel pathway and mechanism of the flocculation need to be investigated in future studies.

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311 Transcriptional Activity and Protein Levels for Horizontally Acquired Genes in Yeast Reveal Hallmarks of Adaptation to Low Nitrogen Content

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In the past decade, sequencing large cohorts of S. cerevisiae revealed the landscape of genomic regions acquired by Horizontal Gene Transfer (HGT). The genes acquired during the HGT process have shown to play important roles in yeast adaptation to the fermentation process, improving nitrogen and carbon sources utilization. However, the functional characterization of these genes at the molecular level has been poorly attended. In this work, we carried out a systematic analysis of the promoter activity and protein expression level for 30 genes contained in three HGT regions commonly known as regions A, B and C. In three strains (one for each region), we used the luciferase reporter gene and the mCherry fluorescent protein to quantify the transcriptional and translational activity of those genes, respectively. We assayed the generated strains in four different culture conditions, showing low levels of transcriptional and translational activity across the environments. However, we observed an increase in protein levels under low nitrogen culture conditions, suggesting a possible role of the horizontally acquired genes in the adaptation to nitrogen-limited environments. Furthermore, since the strains carrying the luciferase reporter gene are null mutants for the horizontally acquired genes. We assayed the fermentation kinetic and growth parameters (latency time, growth rate and efficiency) in this set of deletion strains. The results showed that four horizontally acquired genes influenced the fermentation rate (Vmax) and fifteen of them affected the growth parameters. Altogether, our results provided molecular and phenotypic evidence highlighting the importance of horizontally acquired genes in yeast adaptation, especially under nitrogen-limited fermentation conditions.

312 CRISPR interference technology for development of more tolerant industrial yeast strains

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Second generation bioethanol using lignocellulosic biomass as raw material is a promising alternative to bioethanol produced from sugar-based feedstocks. In addition to sugars, lignocellulosic hydrolysates also contain inhibitors that impair microbial growth. One way to tackle the low productivities is to develop new strains with increased tolerance towards inhibitors.Over the past few years, different CRISPR technologies have been developed to accelerate the construction of new strains. The CRISPR interference (CRISPRi) technology utilizes a catalytically inactive Cas9 (dCas9) to modulate the expression of genes targeted by a sgRNA, allowing the alteration of essential genes and the manipulation of multiple traits without altering the target sequence.

In the present work, our goal was to use CRISPRi to improve the inhibitor tolerance of a polyploid industrial yeast strain. As a proof of concept, the expression of a gene encoding a fluorescent protein was modulated using dCas9 with different activation or repression domains. Changes in fluorescence were measured by flow cytometry and changes in expression were verified by qPCR, validating the use of CRISPRi for alteration of gene expression in an industrial yeast strain. Subsequently, several genes previously identified to be involved in inhibitor tolerance were selected as targets for CRISPRi. The performance of the novel strains during growth in the presence of different inhibitors was analysed in a high-throughput platform, leading to identification of strains where the altered gene expression led to altered tolerance.

313 eIF5A depletion impairs oxidative phosphorylation

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Eukaryotic translation factor 5A (eIF5A) is a highly conserved protein and contains the unusual and essential posttranslational modification that generates the hypusine residue. Although eIF5A was originally described as a translation initiation factor, more recent data have demonstrated that eIF5A interacts with elongating ribosomes and facilitates the translation of specific tripeptide motifs and also stimulates translation termination. eIF5A has been associated with several cellular functions, but it is still not clear the impact of eIF5A function upon different protein levels and to which extent the depletion of some proteins is related to eIF5A mutants phenotypes. In order to better understand the influence of eIF5A on the translational control of gene expression, it was performed a large-scale proteomic screening using the ORF-GFP collection of Saccharomyces cerevisiae and the eIF5A mutant hyp2-3, using the Synthetic Genetic Array methodology. Fluorescence intensities from each individual colony were assayed using a scanning fluorimager to reveal the differential GFP expression. Gene Ontology analysis of the proteomic profile identified enrichment of cellular processes previously involved with eIF5A, such as regulation of cell cycle and translation. Interestingly, an important downregulation of specific proteins of mitochondrial complexes was also observed. Finally, we demonstrate that yeast eIF5A mutants have defect in mitochondrial oxidative phosphorylation. eIF5A might be regulating the translation of specific mitochondrial proteins, which mostly are encoded in the nucleus, synthesized in the cytoplasm and posttranslationally imported into mitochondria as unfolded peptide or cotranslationaly translocated into mitochondria.

314 Regulation of Development of Structured Colony Biofilms.

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On semisolid agar surfaces, yeast Saccharomyces cerevisiae develop multicellular three dimensional structures that exhibit complicated internal architecture and are formed by differentiated cells. Smooth colonies formed by laboratory or domesticated yeast strains and more complex colony biofilms often formed by wild strains, represent two major types of these structures. Complexity of colony biofilm structure is influenced by availability of nutrients and varies between highly structured to rather smooth. The structured biofilm consists of aerial part, which is composed of layers of oval cells and internal cavity, and of subsurface agar-embedded invasive part formed mostly by pseudohyphae. Two factors are indispensable for proper biofilm development: presence of Flo11p adhesin and production of extracellular matrix (ECM) filling cavity and intercellular space of biofilm interior (J Cell Biol 194: 679-87, 2011). How development of such a complicated structure is regulated and how colonies can switch between structured and smooth form, are important questions. Recently, we have identified key function of Cyc8p and Tup1p transcriptional factors in this regulation. (PLoS Genet 14: e1007495, 2018). Tup1p and Cyc8p regulate formation of structured biofilm in the opposite manner, being positive and negative regulators of colony complexity, cell-cell interaction and adhesion to surfaces. Cyc8p functions as a repressor of FLO11 gene expression and of other features essential for structured biofilm formation. Tup1p, however, has a dual function - it counteracts Cyc8p's repressor function and, in addition, contributes to Flo11p stability, probably by repressing a gene coding for a cell wall or extracellular protease involved in Flo11p degradation. These regulatory aspects together with environmental factors involved in colony complexity will be discussed. This work was supported by GACR 19-11384S.

315 Framework for Model Assisted Generation of Platform Strains through CRISPR Mediated Fine-tuning of Central Carbon Metabolism

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Saccharomyces cerevisiae is a well-established cell factory used for industrial production of a wide variety of products. There is a need to develop novel platform strains with increased supply of precursors for industrially relevant chemicals. A time-efficient way to identify targets for genetic engineering is conducting high-throughput screenings. Genetically encoded biosensors can ease these approaches by translating the levels of a metabolite into a detectable output. Overexpression and knock-out libraries have been used for high-throughput screenings but identifying optimized fine-tuning setups for gene expression remains challenging. In this study we used a CRISPRi based approach to create a gene expression fine-tuning library targeting genes potentially increasing the malonyl-CoA pool. The selected genes were identified by Flux Balance Analysis (FBA), several gRNAs per gene were identified and a gRNA library was created using dCas9 fused with the VPR activator. The library was combined with a malonyl-CoA biosensor based on the transcription factor FapR from Bacillus subtilis and screened for cells with biosensor mediated increase in fluorescence. The enriched gRNAs were tested for 3-HP production, a direct malonyl-CoA derivative. We were able to establish a new framework for metabolic optimization of cell factories combining computational modelling and high throughput screening tools allowing the generation of novel platform strains with increased precursor supply.

316 Mitochondria in Development and Differentiation of Yeast Multicellular Structures.

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Yeast as other single-celled microbes form different types of organized multicellular structures, colonies and biofilms, in which cells differentiate to subpopulations localized to specific positions and acquiring distinct functions to fulfill specific tasks within the structure. Differentiated colonies of Saccharomyces cerevisiae laboratory strains are smooth and composed of subpopulations of U cells localized to upper layers and of L cells in colony interior (Mol Cell 46:436, 2012; Oxid Med Cell Longev 2013: ID 102485, 2013). U cells are vital, metabolically active and stress resistant cells that decrease respiration and ROS production by their swollen less cristated mitochondria. L cells exhibit decreased vitality and stress resistance and activate hydrolytic mechanisms to release nutrients to feed U cells. L cells are capable of respiration. Differentiated colony biofilms are more complex and composed of aerial part and subsurface roots (J Cell Biol 194: 679, 2011). We have shown recently that development of some U cell- as well as upper and lower L cell- specific features in smooth colonies is regulated by retrograde signaling pathway transferring signal from altered mitochondria to the nucleus (Oncotarget 7: 15299, 2016). Using comparative transcriptomics by NG-RNA sequencing of cell subpopulations isolated from different positions of colonies of different age (Oxid Med Cell Longev 2018: ID 4932905, 2018) and other approaches, we have further characterized spatiotemporal dynamics of changes in mitochondrial functions during colony development, including those connected with ATP synthesis and respiration. Obtained data support our hypothesis that mitochondria play an important role in colony differentiation processes. Furthermore, transcriptomic comparison of two major areas of colony biofilms - aerial and root parts (BMC Genomics 18: 814, 2017), allowed us to compare potential mitochondrial roles between smooth colonies and colony biofilms. This work was supported by GACR 19-09381S.

317 Ribosome Biogenesis: rRNA Modification, Enzymes and Physiological Significance

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Yeast became the major model organism to study the increasing significance of RNA modification on gene regulation, mRNA translation and ribosome activity.

Within the yeast ribosome 10 bases are methylated on different atoms (4 on18S rRNA and 6 on 25S rRNA) and 2 bases are acetylated. In recent years we have identified most of the base modifying enzymes. Whereas 4 rRNA modifying enzymes have no impact on growth [Methyl transferases Bmt2 (Sharma et al., 2013a), Bmt5 and Bmt6 (Sharma et al., 2014) and acp-transferase Tsr3 (Meyer et al., 2016) all other base modifying enzymes are essential (Methyltransferases Dim1 (Lafontaine et al, 1994), Bud23-Trm112 (White et al., 2008; Figaro et al., 2012) Nep1 (Emg1) (Meyer et al., 2011, Wurm et al., 2010) Rrp8 (Peifer et al., 2013), Nop2, Rcm1 (Sharma et al., 2013b) and RNA-acetylase Kre33 (Sharma et al., 2015). Remarkablly, RNA acetylations are unique as Kre33 needs the assistance of box C/D snoRNAs 4 and 45 (Sharma et al., 2017).

Notheworthy, all essential yeast rRNA modification enzymes have human counterparts which complement the function of the yeast enzymes. These include Nep1 (Emg1), Tsr3, Kre33 (NAT10), Nop2 (NSUN1), Rcm1 (NSUN5), Rrp8 (NML). Many of these enzymes are involved in severe human developmental diseases (Amistead et al., 2009, Am. J. Hum. Genet. 84, 728-73), are important tumor markers (Sato et al., 1999) or have severe physiological impacts (Schosserer et al., 2015).

Most of the essential rRNA modifying enzymes (Bud23, Nep1, Nop2, Rcm1 and Kre33) have a dual function as enzymes and structural components during ribosome biogenesis. Recent cryo-EM structures of the 90S small subunit pre-ribosome show that Nep1 and Kre33 are well exposed and of major importance during the maturation of the small subunit.

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318 Cell Region Fingerprinting Improves Tracking Accuracy in Long-term Time-lapse Microscopy to Study the Inheritance of Dynamic Processes.

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The inheritance of dynamical processes in yeast is nowadays studied using automated time-lapse microscopy of growing 2D cell cultures. For example, the inheritance of growth and cell cycle timing as well as their variability under different metabolic conditions can be revisited, extended and combined with analysis of cell cycle regulation. To perform these types of analysis, we need accurate cell tracking and reconstruction of the genealogy. However, despite many published tracking tools, tracking is challenging as cell movement or imperfect segmentation (associating pixels in an image to a cell) can lead to wrong cell to track assignments. In practice, a researcher still needs to manually verify or curate each cell to track assignment because, without ground truth data, it is currently not possible to evaluate if a track is correct.

To address this limitation, we propose a generic measure for tracking performance to identify potentially problematic assignments that a researcher could focus on. We define the fingerprint distance (FPdist), which measures the similarity of fingerprints (FP) derived from the cells and their surrounding between two compared images. A small distance indicates that regions are similar, suggesting good segmentation and uniform cell movement. A large distance indicates that there is either a wrong assignment or a large movement of the cell.

We implemented the concept in a tracker (TracX) that evaluates initial assignments by the FPdist, rejects unlikely assignments, and uses likely assignments to estimate cell motions, leading to potentially refined assignments in the next iteration. We show that TracX increases tracking performance on published data sets from various species as well as our own long-term time lapse data from S. cerevisiae and S. pombe. Additionally, TracX automatically classifies linkages in good and problematic and thereby provides the user with a mean to rapidly ameliorate the quality of the result. To demonstrate that accurate tracking enables new biological insights, we reconstructed the genealogy of growing 2D colony of S. cerevisiae and studied the inheritance of cell cycle timing over multiple generations. TracX is applicable to many cell types and imaging setups because it only requires the segmentation mask and raw microscopy images. Additionally, other trackers could easily incorporate the idea of using the fingerprint distance for assignment evaluation.

319 Proteome Re-Allocation Towards Amplified Translational Machinery as the Limiting Factor for Increased Growth Rate of Amino Acid Supplemented Saccharomyces cerevisiae.

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An increasing focus in systems biology concerns a global cellular constraint on proteome size and allocation of it between important processes. Through computational efforts, the importance of this constraint-based allocation problem has been shown in for example the expansion of genome scale models to include enzymatic abundancies as a driving factor for chemical reactions. In addition, in silico models have also been developed to investigate the relationship between proteome allocation and growth, in order to estimate maximum growth rates of different systems. Hinting towards the possibility of tailoring the proteome for increased growth rate and improved platform strains. To further develop the concept, we investigated proteome allocation differences in wild type Saccharomyces cerevisiae cultivated in minimal media, in bioreactors, with or without supplementation of amino acids. Proteomics samples as well as extracellular amino acid samples were taken from batch cultivations, under both anaerobic and aerobic conditions, and were correlated. From both conditions, the data implies that amino acid uptake allows the cell to re-allocate proteins from amino acid biosynthetic processes into translation, correlating with an increase in growth rate, while other processes remain rather unchanged. In particular, biosynthesis related to methionine production showed a large decrease, compared to other supplemented amino acids, although several amino acids were taken up simultaneously. The possible potential for the cell to directly re-allocate freed up protein into translational machinery components, and thereby increase its growth rate, suggests that proteome engineering by removing non-necessary produced proteins might be a feasible alternative for optimization of platform strains.

320 Investigating the Biological Significance of RNA Polymerase II Transcription Attenuation at the Yeast DNA Repair Gene, DEF1.

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RNA Polymerase II (Pol II) transcribes all mRNAs and many non-coding RNAs in eukaryotic cells. Termination of Pol II activity serves an essential biological function by separating adjacent transcription units and influencing RNA fate and function. In the yeast Saccharomyces cerevisiae, Pol II termination is carried out by cleavage and polyadenylation factor (CPF-CF) and Nrd1-Nab3-Sen1 (NNS) complexes. Premature Pol II termination (i.e. attenuation) contributes to gene regulation, but there is limited knowledge of its prevalence and biological significance. In particular, it is unclear how much crosstalk occurs between CPF-CF and NNS components and how Pol II attenuation is modulated during stress adaptation.

Our lab previously characterized an attenuator in the DEF1 DNA repair gene that contributes to gene repression in the absence of UV damage. Using a plasmid-based reporter gene system, we identified Pol II read-through mutations in a polyadenylation efficiency element (EE) and the HRP1 gene, which encodes the cognate EE-binding-protein Hrp1. The DEF1 attenuator behaved as a hybrid terminator, relying heavily on CPF-CF and Sen1 but without Nrd1 and Nab3 involvement.

To extend our analysis into a more natural context, we have investigated attenuator function at the chromosomal DEF1 locus in a variety of CPF-CF and NNS mutants. In addition, we have used CRISPR-Cas9 to genetically engineer mutations within the DEF1 chromosomal attenuator. We utilized reverse-transcriptase PCR (RT-PCR) to detect Pol II read-through transcripts and Western blot to quantify Def1 protein accumulation. To monitor the biological significance of Def1 overexpression, we combined an attenuator mutation with a constitutively nuclear Def1 mutant, and we monitored cell toxicity in the absence and presence of UV. Overall, we aim to support a biologically meaningful role for attenuator function in repressing DEF1 gene expression in the absence of DNA damage.

321 TORC1 Function and Regulation During Yeast Colony Develepment.

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Saccharomyces cerevisiae laboratory strains form multicellular communities, colonies, in which cells are able to differentiate. We characterized two major cell subpopulations that are formed during colony development and that differ morphologically and physiologically [1, 2]. U cells are characterized by high expression of translation machinery genes, adaptation to nutrient limitations and signaling from mitochondria with lowered respiration. In contrast, L cells upregulate the transcription of many starvation- and stress-responsive genes and those involved in utilization of poor nitrogen sources. U cells activate a unique metabolism, including activation of nutrient sensing pathway TORC1, i.e. the pathway which typically inactive in stationary-phase cells. The evolutionarily conserved TORC1 kinase represents a master growth controller that integrates diverse environmental inputs to coordinate many metabolic processes and plays a role in biosynthesis and proliferation. We tracked TORC1 activity in the colonies using GFP-labeled Gat1p. Gat1p is a TOR-responsive transcription factor and moves to the nucleus upon TOR kinase inactivation. A majority of U cells exhibited cytosolic localization of Gat1p-GFP, while a predominant nuclear localization of Gat1p-GFP was observed in L cells. As expected, the TOR-inactivating drug rapamycin induced Gat1p-GFP relocalization to the nucleus in U cells [1, 2]. In addition to comparing major U and L cell subpopulations, we performed genome-wide transcription profiling by RNA sequencing of cells separated from different areas of colonies and in different developmental phases (6- and 15-day-old colonies). These comparisons revealed large expression differences between individual cell subpopulations [3]. These analyses contribute to identifying metabolic processes potentially regulated by the TORC1 complex. The work was supported by GAUK 912218.

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322 Elucidating the function of alpha-N-terminal protein methylation of Hsp31 and other N-terminal methyltransferase substrates in proteostasis and stress response.

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The post-translation modification, alpha-N-methylation, has been detected in proteins for over 40 years. However, the function of this cryptic modification has only recently been described in eukaryotic cells in an under-represented set of proteins. We are using Saccharomyces cerevisiae as a model for investigating the N-methyltransferome, which has only one reported alpha-N-methyltransferase. Tae1, is the yeast alpha-N-methyltransferase that recognizes a consensus N-terminal motif sequence, M-X-P-[K/R], similar to its human homologues NTMT1/2. NTMT1/2 have important functions in cell division and are implicated as a cancer targets. Tae1 has previously been implicated in methylation of ribosomal proteins and regulating protein translation. Bioinformatic analysis indicates that 45 proteins have the consensus motif recognized by Tae1 including heat shock protein 31 (Hsp31) and its paralogues (Hsp32, Hsp33 and SNO4/Hsp34). The yeast chaperone, Hsp31, is a multitasking protein that adopts a homodimer conformation and has functional similarities to Parkinson's disease protein, DJ-1. Hsp31 is involved in multiple cellular functions including oxidative stress sensing, protein folding, proteasome degradation, and deglycase enzyme activity. Our lab previously demonstrated that Hsp31 expression is induced by oxidative stress and alpha-Syn mediated proteotoxic stress. In recent experiments, we describe the novel alpha-N-methylation of Hsp31 and other substrates. We have verified the presence of mono- and di-methylation on Hsp31 and made single amino acid mutations on the consensus motif of Hsp31 with varied methylation potencies to investigate the function of this modification. Preliminary in vitro biochemical assays suggested that loss of Hsp31 methylation leads to increased chaperone activity. We also observed that Tae1 deletion strains have increased heat shock resistance. These studies suggest that alpha-N-methylation by Tae1 decreases intrinsic heat shock resistance and maybe a key regulator of cellular proteostasis. Further studies are aimed at confirming the alpha-N-methylation level of each Hsp31 mutant and establishing the relevance between alpha-N-methylation level and different Hsp31 functions. In this project, we describe novel findings that shed light on the N-methyltransferome of a eukaryotic model organism and its implications in controlling cellular proteostasis.

323 The Hsp90 Co-chaperone Sgt1 is a Novel Player in Cytosolic and ER Protein Quality Control and Aging

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Buildup of misfolded and potential toxic protein conformers are prevented by chaperone-assisted refolding and/or protease-dependent degradation but how these two systems of protein quality control (PQC) are coordinated is not fully understood. In this work, using a genome-wide screen, we identified the essential Hsp90 co-chaperone Sgt1 as a hitherto unknown member of general PQC linking folding and degradation activities by participating in the degradation of misfolded proteins both in the cytosol and ER. The Sgt1-dependent pathway of protein degradation acts in parallel with the ubiquitin ligase (E3) and ubiquitin chain elongase (E4), Hul5, and overproduction of Hul5 can partly suppress PQC defects, poor growth, and accelerated aging of cells with reduced Sgt1 activity.

Furthermore, Sgt1 is required for the clearance of aggregates during stress and ageing, the formation of distinct protein inclusions and their efficient clearing.

324 Ca2+/Calmodulin, Calcineurin and metacaspases : The 3-Cs of Protein Quality Control in Yeast.

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Calcium dependent apoptosis-inducing protein in yeast is known as metacaspase (MCA1). MCA1 was recently shown to act as a key player also in maintaining proteostasis under stressful conditions such as heat shock. MCA1 interacts with Calmodulin (CMD1), Sir2, Myo2, YDJ1 and Hsp104. However, molecular regulators controlling the executioner-related role of MCA1 remains elusive. Here we report that calcium overload leads to generation of toxic protein aggregates in the yeast Saccharomyces cerevisiae and that this process is Calmodulin dependent. Partial inhibition of the calcium-dependent Calcineurin (CN) by tacrolimus protected the yeast cell against protein aggregates triggered by overexpressing a version of MCA1 displaying constitutively high protease activity. Therefore, our results suggests that caspase-like activity of MCA1, at least in part is regulated by the Ca+2-dependent Calcineurin phosphatase. Phosphatase activity of Calcineurin is tightly regulated by various Ca+2 ion channels and how the calcium sink effects caspase function is presently under investigation. Age related loss of calcium homeostasis and cytoplasmic calcium overload has been implicated in several apoptotic conditions in the eukaryotes. Based on these observations, the ultimate aim of this investigation is to elucidate the key regulatory events triggering/combating calcium overload during Protein Quality control (PQC) breakdown and MCA1 induced cell death.

325 "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 50 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.

326 3D Printing of Yeast-Laden Hydrogels for Improved Bioprocess Efficiencies.

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3D printed yeast-immobilized hydrogels coupled with bioreactors provide a novel platform for flow biochemistry bioprocesses. Ideally, the cells embedded within the hydrogels are viable, and their metabolic activity could be utilized to transform substrates into pharmaceuticals or other chemical feedstocks of interest. We have developed a process that utilizes multi-stimuli responsive hydrogels to encapsulate and print yeast cells. These yeast-laden hydrogels have potential to actively transform glucose into ethanol over the period of several months and allow decoupling of growth and metabolic activity of the cells. Possibility to immobilize cells in a leakage-free system can have a multitude of advantages compared to conventional bioprocesses, as the system allows (i) keeping cells metabolically active for an extended period of time, (ii) reducing cellular growth, allowing more substrate to be converted into the desired product, (iii) using co-cultures in desired ratios throughout the entire process, (iv) reduced costs for downstream processes, and (v) increased flexibility of the system.

Immobilized yeast cells have been studied using three different polymer compositions over an extended period of time. The best performing composition was selected for further analysis, where the growth of the cells and production profiles have been characterized under aerobic and anaerobic conditions. The performance of several producer strains have been characterized and compared to the suspension cells in case of various products. In this presentation, I will give an overview of the challenges and potential of using 3D printing for cell immobilization in biotechnology processes as well as for other potential applications.

327 Production Of Muconic Acid And Protocatechuic Acid From Lignocellulosic Feedstock

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Converting Saccharomyces cerevisiae into a microbial cell factory by engineering new metabolic pathways facilitates the establishment of a sustainable bio-based industry. Muconic acid (MA) serves as an interesting platform chemical for the production of bio-polymers with novel characteristics due to its two double bonds, which allow a great variety of additional modifications in the polymer. In the past, Saccharomyces cerevisiae has been tailored for the production of muconic acid by expression of a heterologous pathway consisting of three different genes (MApw). As such, 3-dehydroshikimate, an intermediate of the shikimate pathway, responsible for aromatic amino acid biosynthesis, is converted via protocatechuic acid and catechol into muconic acid. We are applying a similar strategy for production of muconic acid, for the first time in an industrially relevant pentose-utilizing S. cerevisiae strain, which allows to use lignocellulosic biomass as substrate. Feedback inhibition by aromatic amino acids on the first enzyme of the shikimate pathway was abolished and co-factor production was increased for more efficient conversion of protocatechuic acid (PCA) to muconic acid. Finally, ethanol production was eliminated by replacing each of the pyruvate decarboxylase genes PDC1, PDC5 and PDC6 with a copy of the heterologous muconic acid pathway. This resulted in titers of about 2300 mg/L and 1300 mg/L of muconic acid and PCA respectively. Moreover, alternative isoforms of the protocatechuic acid decarboxylase (PCAD) enzyme were inserted to allow for a more efficient conversion of PCA towards MA. This further increased the final titer of muconic acid to 2600 mg/L. In addition, other modifications such as inserting extra copies of the central pathway enzymes TKL1 and the feedback resistant ARO3K222L are explored to increase substrate availability for the shikimate pathway. In a similar way, expressing only 3-dehydroshikimate dehydratase for production of PCA as final product resulted in a titer of 3700 mg/L.

328 In-silico Yeast Chassis Design for Accelerated Cell Factory Development

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Saccharomyces cerevisiae is a widely used microorganism for industrial biotechnology that has great potential to replace traditional petrochemical synthesis. Optimization of cell factories for production of different biotechnological products is still a cost and time inefficient process. Availability of pre-optimized yeast chassis cells, with improved precursor supply, will overcome such hurdles. Building upon this premise, we have developed a framework for rational design of chassis strains combining genome-scale metabolic models with a multi-objective metaheuristic approach. As proof-of-concept, we have generated pre-optimized chassis yeast cells for enhanced production of dicarboxylic acids. Several multi-gene deletion strains, including the chassis cell and the final producer strains, were implemented and experimentally tested. As predicted, strains encompassing the chassis backbone exhibited higher titers of respective targeted compounds than those containing merely the intuitive gene deletion(s). Taking advantage of the growth-product coupled chassis design, adaptive laboratory evolution (ALE) was employed to the best producing strains. Finally, evolved strains were characterized using a systems biology strategy. In this work, we show that modular design strategies combined with ALE can contribute to accelerate cell factory development for production of natural compounds.

329 WGS-based Identification of Recurrent Mutations That Confer Adaptation to Translation Termination Defects in Yeast.

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Baker's yeast Saccharomyces cerevisiae is a well-known eukaryotic model organism that is widely used to study mechanisms of fundamental cellular processes. One example of such processes is the termination of translation, a crucial stage in protein synthesis. Termination of translation in yeast, like in most eukaryotes, is controlled by two main release factors, eRF1 and eRF3, encoded by the essential SUP45 and SUP35 genes, respectively. Previously we showed that even though these genes are essential yeast cells can maintain viability upon nonsense mutations in them (sup35-n and sup45-n). Interestingly, viability of the cells harboring these mutant alleles is increased after growth in the absence of wild-type allele, suggesting that additional mutations may arise during the first stage of selection. In this study we set off to identify such mutations and characterize their role in conferring cellular adaptation to translation termination defects.

We first constructed a chromosome-level de novo reference genome assembly of one yeast strain from the Peterhof Genetic Collection, 1A-D1628, using data from Oxford Nanopore Technologies (ONT) MinION sequencer. The assembly was further polished using both raw ONT data and paired-end Illumina reads. The resulting reference assembly contained 23 contigs, including all but one yeast chromosomes assembled in a single contig. We then used the obtained assembly as the reference to search for genetic variants present in 100 yeast clonal cultures obtained by substitution of the wild-type allele of either SUP45 or SUP35 gene for the respective nonsense mutant copy. We identified 559 mutations arising after plasmid shuffling procedure, 428 of which were uniquely present in strains resulting from substitution to the mutant allele. 100 of such mutations occured 3 or more times in strains that harbored the mutant allele of SUP35 or SUP45. The role of these mutations in survival of yeast cells lacking functional termination factors is currently under examination. Dissection of the adaptive mutations that help cells survive upon severe translational defects would provide new insights into the mechanisms of translational regulation and may suggest new strategies for disease therapy.

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330 Multilayered Control of Protein Turnover by TORC1 and Atg1

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Autophagy is a constitutive as well as a stress-induced cellular turnover pathway. Autophagy related (Atg) proteins regulate autophagy and orchestrate lysosome/vacuole-dependent turnover of macromolecules and organelles. In the budding yeast Saccharomyces cerevisiae 42 Atg proteins are known to play a role in the vacuolar targeting of macromolecules to be catabolized. In eukaryotes, the Target of Rapamycin Complex 1 (TORC1) is a conserved hetero-multimeric kinase complex responsible for the regulation of crucial cellular functions, such as growth and metabolism, acting on numerous processes like protein synthesis, ribosome biogenesis and autophagy. TORC1 regulates these processes by integrating various signals that mediate nutrient levels to match growth with the metabolic state of the cell. The signals impinging on TORC1 vary from basic building blocks like amino acids, which are sufficient to activate TORC1 in unicellular organisms like budding yeast, to hormones and growth factors, which, combined with amino acids, are primarily important to activate TORC1 in higher eukaryotes. In yeast, TORC1 represses autophagy by phosphorylation of Atg13, a subunit of the Atg1 kinase complex, which is critical for the initiation of the autophagic process. Under starvation conditions, autophagy is induced through the inactivation of TORC1 and consequently dephosphorylation of Atg13 and activation of the Atg1 kinase, which in turn phosphorylates and activates downstream effectors that play critical roles in autophagy. To elucidate additional potential levels of crosstalk between TORC1 and Atg1, we analyzed, using quantitative proteomics, the overall changes in the phosphoproteome following TORC1 inhibition and/or Atg1 inactivation. Our respective data indicate the existence of an extended network of reciprocal and autoregulatory loops involving both TORC1 and Atg1. To further corroborate some of our data, we also individually purified all of the yeast Atg proteins and subjected them to in vitro kinase assays with purified TORC1 and Atg1 kinases. This allowed us to identify hitherto unknown Atg1 target residues in Atg proteins, which we demonstrate are physiologically relevant for proper control of autophagy in vivo.

331 Functional Characterization of Putative Acetate Transporters and Channels in the Human Fungal Pathogen Candida glabrata.

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Successful human colonizers such as Candida species have evolved distinct strategies to survive and proliferate within the human host. These strategies include sophisticated mechanisms to rapidly adapt to a diverse range of environmental stresses and assimilate the available nutrients. For instance, during gastrointestinal and vaginal colonization, where glucose is scarce, alternative carbon sources such as acetate or lactate are particularly abundant and may support the growth and the proliferation of C. glabrata cells. Our studies have demonstrated that the presence of these alternative non-fermentable carbon sources influence biofilm formation, antifungal drug resistance and immune recognition. Additionally, there is evidence that putative acetate transporters and channels have an important impact on these processes. Here, we provide a detailed view on the role of putative C. glabrata acetate transporters during carbon adaptation. Our data support the view that adaptive responses of Candida cells to alternative carbon sources affect their virulence, through multifarious mechanisms.

332 Yeast Chemical Genomic Approach for Target Identification.

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Target identification of bioactive compounds is critical for drug discovery, but it still remains a major challenge. We developed a robust chemical genomic platform for precise target identification by constructing DNA-barcoded yeast mutant libraries that were amenable to highly multiplexed next-generation sequencing for rapid assembly of chemical-genetic profiles. Our platform includes:

1) A quantitative barcode analysis system for measuring the fitness of ~1,000 essential gene heterozygous diploid mutants (HET) (for drug-induced haploinsufficiency), ~1,000 temperature sensitive haploid mutants for the essential genes (TS) and ~3,000 non-essential gene deletion haploid mutants (WG; whole genome) (for comparisons to genome-wide yeast synthetic genetic interaction data).

2) An orthogonal pipeline to directly identify target genes using next-generation whole-genome sequencing and complementation cloning with the barcoded yeast ORFeome overexpression library (MoBY-ORF) of ~1,000 strains that confer drug resistance.

3) A CRISPR-based mutant generation approach for validating drug-target interactions through targeted cytidine deamination.

Taking advantage of a valuable collection of natural products from the RIKEN Natural Product Depository (NPDepo), we identified compounds that target specific cellular functions with therapeutic potential. We identified compounds with strong and specific drug-induced haploinsufficiency interactions with yeast essential genes. These compounds were prioritized for validation using spontaneous drug-resistant mutant analysis, which often identifies a mutation directly in the target gene. This approach allowed us to identify precise gene targets for novel compounds. For example, NPD6433 has a precise HET interaction with FAS1 (fatty acid synthase), and a drug-resistant mutant for NPD6433 shows a non-synonymous single nucleotide polymorphism (SNP) in the same gene. The non-synonymous SNP occurs in an amino acid that is in close proximity to the flavin mononucleotide (FMN) cofactor site of Fas1. Supplementation with both fatty acids and FMN rescues NPD6433 toxicity, so it is likely that NPD6433 directly targets Fas1.

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333 Simultaneous Quantification of mRNA and Protein in Single Cells Reveals Trans-acting Genetic Variation.

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Heritable variation in gene expression is a key source of phenotypic diversity and evolutionary change. Most of this variation arises from trans-acting DNA variants located on different chromosomes from their target genes. Previous work reported trans-acting variation that specifically affected mRNA or protein levels of a given gene. However, this conclusion rests on studies with low statistical power, conducted at different times, with different experimental designs, and in different environmental conditions. To fully understand regulatory genetic variation, we need a strategy that combines high statistical power with simultaneous readouts of mRNA and protein.

To address this challenge, we developed a system for quantification of mRNA and protein levels in single, live cells of Saccharomyces cerevisiae. We tagged the C-terminus of genes of interest with green fluorescent protein (GFP), followed by a polyA tail and a CRISPR guide RNA sequence flanked by two ribozymes. After transcription, the ribozymes release the guide RNA, which directs a deactivated Cas9 protein fused to a transcriptional activation domain to drive expression of an mCherry reporter gene. Thus, mCherry serves as a readout of mRNA abundance, while the GFP tag allows measurement of protein levels in the same cell.

This system enabled us to use deep bulk segregant analysis to map the genetic basis of gene expression variation. For ten genes with diverse functions and regulatory architectures, we generated millions of recombinant cells by crossing two yeast isolates. We used fluorescence-activated cell sorting to collect pools of thousands of these cells with high or low mRNA or protein levels. Whole-genome sequencing of these pools revealed loci that affected mRNA or protein with high statistical power.

Across the ten genes, we identified 97 trans-acting loci that affect gene expression. Although we measured mRNA and protein at the same time in the same cells, we detected clear discrepancies. Less than 30% of loci significantly affected mRNA and protein for the same gene with concordant direction of effect. Nearly half of loci strongly influenced the protein level of a given gene but showed no effect on its mRNA. Four loci influenced both mRNA and protein of the same gene strongly, but in opposite directions.

This work revealed complex and distinct trans-acting genetic influences on protein and mRNA levels, with important implications for the diversity and evolution of gene expression.

334 Beyond S288C: Incorporating Genomic Sequence Information from Large-Scale S. cerevisiae Population Surveys into SGD.

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The Saccharomyces Genome Database (SGD; www.yeastgenome.org) began 25 years ago as a repository of the whole genome “reference sequence” of the S. cerevisiae S288C lab strain — the first sequenced eukaryotic genome. Recent advances in genome sequencing technology have led to an explosion in the number of whole-genome sequences of S. cerevisiae strains isolated from a wide variety of geographical locations and environmental niches, with over 1500 different strain sequences currently deposited in public databases, and many more certain to be added in the coming months and years. Large-scale genomic comparisons using these data allow exploration of the genetic and phenotypic diversity of natural populations of yeast, helping us understand both the origins and the present-day distribution and standing genetic variation of this important organism. At SGD we see challenges and opportunities in the process of incorporating this large amount of genomic information. We present various ways to store and display strain-specific genomic information, and to present information gleaned from comparisons across sequenced strains. These include the addition of Locus Pages for open reading frames (ORFs) not found in the S288C reference genome, the identification and labeling of “core” ORFs (i.e., those shared by virtually all whole-genome sequenced strains) vs. “variable” ORFs, and the display of sequence variation in ORFs across many strains. We also describe “Strain Pages” for sequenced strains, containing relevant details on environmental niche, phenotypes, phylogenetic clade and links to the genome sequence. We hope that the addition of these information categories will be of great use to the yeast community, providing easy access to information about strain variation as well as about the ecology and population dynamics of S. cerevisiae.

335 Exploring S. Cerevisiae Domestication From The Analysis Of Their Genome And From An Experimental Evolution Approach In The Grape Must.

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Saccharomyces cerevisiae strains isolated from wine, rum, bread, cheese, or oak present a wide phenotypic divergence (1, 2), while we have little information about the genetic bases. In order to decipher the genetic basis of the adaptation to anthropogenic environments we have sequenced the genome of 82 strains isolated in these niches and shown that adaptation to anthropogenic environments results from the acquisition of niche specific genes by horizontal gene transfer, gene amplification, and the selection of specific alleles (2).

In order to evaluate how genetic variation can participate to the adaptation to the grape must environment, we used an experimental evolution. We built recombinant populations with different amounts of standing variation using strains from wine and various origins, and grew them for 24 fermentations interspersed with nine population-wide sexual cycles. The phenotyping of these population revealed that recombinant population including wine alleles presented the best fitness in the grape must, higher than that of recombinant population devoid of wine alleles. The analysis of the genomes of evolved populations at the end of the experimental evolution revealed shifts in allelic frequencies pointing to regions containing genes with key functions. Some regions were shared with those detected from the genomic diversity analysis.

These two strategies provide complementary visions of the evolution of wine yeast in its environment leading to its domestication.

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336 In Vivo Dynamics and Assembly of the Ssn6-Tup1 Global Corepressor Complex upon Glucose Repression.

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The Ssn6-Tup1 complex of the budding yeast Saccharomyces cerevisiae associates with a number of transcription factors to regulate gene expression. The Mig1 transcription factor of the glucose repression pathway recruits Ssn6-Tup1 in order to repress target genes under high glucose conditions. However, very little is known about the dynamic behaviour of the functional co-repressor complex, how its assembly is established and regulated. The role of the dynamic interactions between the individual subunits within the complex in the native cellular environment remains to be elucidated. We use high-speed single-molecule super-resolution Slimfield microscopy to address questions related to the molecular architecture, dynamics and kinetics of the co-repressor complex and its components in single living cells.

Our data provide insights into dynamics and cellular localisation of interactions between the Ssn6-Tup1 complex and Mig1 under different extracellular glucose conditions. We also determined apparent protein stoichiometry and diffusion coefficients of the co-repressor complex components and how it is related to the glucose presence in cellular microenvironment. Alternating Laser Excitation approach allowed real-time imaging of protein-protein interactions within living cells. Here, we present a model of the Mig1 – Ssn6-Tup1 complex mobility, assembly and dissociation, and recruitment to DNA, thus, shed some light on the dynamics of gene regulation in glucose repression pathway.

337 Transcriptome Analysis Reveals the Moon-Lighting Role of Hsp31p in the Budding Yeast Cell Defense Against Fermentation-Related Stresses.

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Saccharomyces cerevisiae Hsp31p protein belongs to the ubiquitous DJ-1/ThiJ/PfpI family. 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.

Comparative transcriptome analysis of ethanol and acetate-stressed cells revealed that the absence of Hsp31p affects numerous molecular functions and biological processes of S. cerevisiae cells. Notably, some of them are localized at the cell periphery. Interestingly, Hsp31p whose intracellular localization was a matter of debate turned out to be localized in the periplasmic space as well. This makes Hsp31p another piece of armor in yeast cell armament against environmental stresses, deployed at the first line of defense, the periplasm.

Although Hsp31p is known as an enzymatic protein, our transcriptomic data suggest its role as a component of ethanol stress response system, because in hsp31Δ cells the reaction to this stress is perturbed. In particular, stress-induced protein synthesis arrest is compromised in these cells. Our results reveal Hsp31p as yet another moon-lighting protein, being an enzyme decomposing toxic compounds and the suggested environmental stress-signaling protein. Both functions are compatible with recently established periplasmic localization of this protein.

338 Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae

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The replication checkpoint is essential for accurate DNA replication and repair, and maintenance of genomic integrity when a cell is challenged with replication stress. An important feature of the replication checkpoint is the regulated localization of many cellular proteins in the budding yeast Saccharomyces cerevisiae. This replication checkpoint is initiated by the essential Mec1 and Rad53 kinases in budding yeast. Here, we assessed the roles of the replication checkpoint kinases in regulating protein localization during drug-induced replication stress. We employed a high-throughput confocal microscopy platform to monitor movements of GFP-tagged proteins following treatment with methyl methanesulfonate (MMS). While canonical checkpoint signaling regulates one-third of observed protein movements, we find an uncharacterized role of the Rad53/CHK2 kinase in regulating protein localization. This suggests a role for Rad53 in the replication checkpoint that does not involve Mec1; a feature largely unexplored. Together, checkpoint kinases regulate a subset of protein localizations and our results have revealed proteins with novel roles in the replication checkpoint.

339 Coordinate regulation of the cell cycle and re-fertilization blocks ensures ploidy maintenance in fission yeast.

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To ensure genome stability, sexually reproducing organisms strictly ensure that fertilization occurs only between two haploid gametes and meiosis only in diploid zygotes. Fission yeast gametes arrest the cell cycle in G1, fuse with a partner and immediately enter meiosis. We recently showed that zygotes swiftly repress mating post-fertilization to prevent polyploid formation by reconstituting a bipartite transcription factor that drives the expression of the mei3 gene (Vjestica et al., Nature 2018). We now show that recurring fertilizations correlate with the zygotic cell cycle arrest and that forced cell cycle progression alleviates re-fertilization. Our results show that the zygotic Mei3-Pat1-Mei2 signaling cascade, which was considered to specifically induce meiosis, is in fact wired to push cells through mitosis when meiosis is impaired. Both Mei3 and Mei2 independently activate the cyclin-dependent kinase through distinct regulation of cyclins. Mei3 is essential for the zygotic G1-S transition and its overexpression triggers this transition in gametes. Surprisingly, Mei2 employs G1-S cyclin Cig2 not only to drive genome duplication but also to ensure robust meiotic divisions. Consistently, live imaging revealed that Cig2 levels peak at S-phase and both meiotic division, which defined one of four expression patterns we find for zygotic cyclins. Our results suggest that Mei3 and Mei2 branches of zygotic signaling quantitatively superimpose to achieve high activity of the cyclin-dependent kinase, which is required for meiotic progression. Taken together our results show how sexual reproduction imposes a cell cycle regulation that ensures both robust re-fertilization blocks and meiotic progression.

340 Functional and Evolutionary Characterization of Yeast Xylose Transporters Through Comparative Genomics and Machine Learning

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Continuous use of fossil fuels as an energy source has reached a worrying point concerning the consequences of greenhouse gas emissions to climate change. For these reasons many countries, including Brazil, have been studying alternative energy sources, one of those being second generation ethanol (2G) produced from biomass, mainly sugarcane bagasse and straw which are left in the fields after harvesting. Even though 1G ethanol production from molasse is highly productive, some challenges remain for 2G, one of them being that yeast strains cannot ferment xylose, one of the main sugars in 2G feedstock, for lack of good xylose transporters. Even though many studies have been made trying to characterize xylose transporters we still don't know what patterns make one transporter capable of carrying this sugar. In an attempt to better understand these unknown patterns we have taken two approaches: one based on a comparative genomics study of 180 yeasts, searching for patterns of positive selection on transporter genes of xylose-consuming or fermenting species that may indicate adaptations to this phenotype, and machine-learning, searching for patterns in the linear sequence and 3D structure of known xylose transporters against non-xylose transporters, finding the most important features to this phenotype and predicting potential candidates to test in wet-lab. Our comparative genomics approach resulted in 3 transporter families and 2803 transporter genes obtained through Orthofinder. For each family we inferred phylogenies through IQTree and marked fermenter and consumer species' proteins for positive selection analysis using MEME, implemented in the HYPHY package. We report for the first time, to our knowledge, evidence of positive selection in transporter proteins. Additionally, after reducing the unbalance of our 400 yeast transporter dataset (as there are only 25 xylose transporters reported in literature) our machine-learning model based on the XGBoost gradient boosting decision trees algorithm predicted 13 important features out of 30 thousand we initially extracted, including one based on an HMM calculated for the pore of transporters. With this model, we tried predicting from the 2803 transporters, resulting in 25 potential candidates for wet-lab testing. These results show the potential of these approaches for choosing candidates for industrial applications and providing robust strains for biotechnological applications after rational genetic engineering.

342 Absence of the Prion-like Domain of the mRNA Decapping Activator Lsm4 Selectively Reduces mRNA Stability Under Stress

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P Bodies are evolutionary conserved RNA granules concentrating mRNA degradation factors, translational repressors and non-translating mRNA in cytoplasmic foci. We present data that a mutant unable to form P bodies affects mRNA decay rates and enforce directionality on mRNA degradation in yeast. This mutant lacks the mRNA decapping activator Edc3 and the Q/N rich C-terminus of Lsm4, part of the Lsm1-7 mRNA decapping activator complex. We have also obtained genome-wide data that mRNA stability is significantly reduced for the P body mutant, but also for the lsm4ΔC mutant, which does not localize Lsm1-7 to P bodies. These mutants also simultaneously have an increased stability for environmental stress response mRNAs that are normally degraded in response to stress. These data suggest a role for the Lsm1-7 complex in orchestrating the mRNA degradation response during environmental stress.

343 High Throughput Screening for Enhanced Production of Natural Compounds in Yeast

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Microorganisms are becoming increasingly important as so-called cell factories, engineered to produce compounds of interest. These cell factories have great potential to provide a sustainable alternative to production processes depending on fossil resources.

Metabolic engineering has become a powerful tool to harness the potential of microorganisms and turn them into cell factories. The first step is to either identify an organism that produces the chemical of interest naturally, or to introduce a corresponding production pathway into the cell.

In both cases it is generally necessary to perform additional engineering steps in order to boost production, since both the synthesis pathway and the overexpression of enzymes consume considerable amounts of cellular resources and may in fact imbalance the entire metabolism. Therefore, it is paramount to identify bottlenecks and find strategies to counteract or remove them.

Here we use Saccharomyces cerevisiae as a model organism, into which we introduced the production pathways for different, natural compounds. The products of interest were selected, so that their biosynthetic pathways diverge from different parts of host metabolism, and include, so far, vanillin glucoside and β-carotene.

As a first optimization step, we performed a cofactor switch, i.e. we replaced NADPH-dependent enzymes with their naturally occurring NADH-dependent isoforms. This modification would allow to shift the cellular cofactor balance in favor of NADPH, which can then be used by the heterologous pathway.

Now we aim to identify targets to further boost the production of the molecules of interest. To achieve this goal, we introduced the above mentioned pathways into a deletion library of S. cerevisiae, generating more than 200 modified strains. To screen the generated library with respect to production and physiology, we needed to implement a high throughput, small-scale cultivation and screening process.

344 The Role Of MAPK HOG1 In The Tolerance To Saline Stress In Debaryomyces hansenii.

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Debaryomyces hansenii has been studied for its capability to adapt to saline stress. Originally isolated from marine water, can also be found in salty foods. It can accumulate high Na+ concentrations, and its adaptation has been attributed to some improved enzymes, different metabolic routes, or a combination of processes converging to cope with saline stress.

The HOG (high osmolarity glycerol) pathway mediates a significant part of the response of yeast cells to a hyperosmotic shock, since it is required for the stimulated expression of more than 100 genes. The osmo-induced genes include GPD1 and GPP2, which encode enzymes involved in the production of glycerol, the main osmolyte accumulated by yeast cells.

It is of our interest to know the participation of the general Hog1 pathway in the saline resistance of D. hansenii, but this yeast is hard to transform. Several groups of colleagues have tried to delete some genes without success. We contacted Dr. Papon in France since he and his group have designed some plasmids to transform yeasts from the CTG clade.

The yeasts from the CTG clade translate the codon CTG in serine instead of leucine. The reassignment of the CUG codon from Leu to Ser occurs in at least 75 Candida species and in Pichia stipitis, D. hansenii and Lodderomyces elongisporus.

Using the p244 plasmid which encodes a SAT1 gene for resistance to nourseothricine and a yeYFP1 gene for yellow fluorescent protein with optimized codons for expression in D. hansenii, we succeeded in obtaining a hog1 mutant of this yeast.

Here, we report the obtaining and characterization of the hog1 mutant from D. hansenii.

347 Intracellular phosphate reception and signaling: A novel homeostatic system with roles for an "orphan" organelle?

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Cells face a phosphate challenge. Growth requires a minimal concentration of this limiting resource because intracellular phosphate (Pi) is a compound of nucleic acids and modifies most cellular proteins. At the same time, cytosolic Pi may not rise much, because elevated cytosolic Pi can stall metabolism. It reduces the free energy that nucleotide triphosphate hydrolysis can provide to drive energetically unfavorable reactions.

Our group tries to elucidate how cells strike this critical balance. We characterize a novel pathway for intracellular phosphate reception and signaling (INPHORS), which may regulate cytosolic Pi homeostasis by coordinating multiple SPX-domain-containing proteins for import and export of Pi, and for Pi storage in acidocalcisomes. Acidocalcisomes are conserved but very poorly understood organelles. We propose that their role in buffering cytosolic Pi concentration is one reason for their evolutionary conservation.

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348 Species-wide survey of conditional gene essentiality across yeast natural populations

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The same mutation does not always cause the same phenotype in different individuals due to differences in their genetic backgrounds. Such background effect may constitute an inherent feature of biological traits, complicating our ability to predict phenotypes from genomic information. In model systems, a classic example of background effect is conditional gene essentiality, which occurs when the loss-of-function of a gene causes lethality in one background but not another. Between two yeast strains, S288c and ∑1278b, ~1% of all genes were conditional essential. Understanding the genetic basis of conditional essentiality offers a great opportunity to dissect the origin of background effects - a potential source of “missing heritability” in complex traits.

Over the past few years, an expanding number of natural yeast isolates in the Saccharomyces cerevisiae species has been completely sequenced. These isolates are originated from various ecological and geographical sources and are genetically diverse, with maximum nucleotide divergence ~1.5%. Taking advantage of these resources in the yeast system, our goal is to survey the entire species for background-dependent phenotypes related to gene deletion mutations. We generated a collection of ~450 strains that are diploid, euploid and homozygous, which were originated from monosporic segregants of over 1,000 wild yeast isolates. To efficiently create gene deletion mutants in these wild strain backgrounds, we developed a CRISPR-Cas9 based plasmid library that allows for one-stop PCR-free gene deletion across the whole genome, for any background of interest across the S. cerevisiae species. Each deletion mutant generated using this method will carry barcodes that allow for precise identification in pool-based strategies. This powerful resource will greatly benefit the yeast community in studying background-specific mutation effects, higher-order genetic interactions and gene-environment interactions.

349 Synthetic biology of yeast S. cerevisiae

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With the rapid development in DNA synthesis and DNA sequencing, DNA assembly and strain engineering start to be the rate limiting step in the pipeline. Moreover, the upcoming wave in synthetic biology automation also demands a rethinking of how we perform DNA assembly and genome editing. Here, a non-enzymatic assembly method that is capable of assembling a 7 kb plasmid from 10 fragments at ~80% fidelity and a 31 kb plasmid from five fragments at ~50% fidelity; and a CRISPR based method that can generate 8-gene disruptions in S. cerevisiae at ~90% fidelity will be introduced. Moreover, a fast genome editing method, in which the Golden-gate reaction mix being directly transformed to yeast with ~90% fidelity on 4-gene disruptions will also be discussed. The two synthetic biology tools described here should be invaluable additions to a synthetic biologist's toolbox.

350 Fluorogen Activating Proteins as a Powerful New Imaging Tool for Quantitative Protein Trafficking Studies in Yeast.

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Recent advantages of genetically encoded fluorescent probes have led to the development of fluorogen activating proteins (FAPs). This technology has two components: a non-fluorescent single chain antibody (SCA) that can be fused to a protein of interest and fluorogens, which are non-fluorescent dye molecules when free in solution. When the SCA and fluorogen bind, there is a 20,000-fold fluorescence increase relative to unbound dye. This level of fluorescence is comparable to standard fluorescent proteins, like GFP. However, the FAP-technology has two major advantages; (1) using either a membrane-permeant or impermeant fluorogen dye we are able to selectively label intracellular proteins from proteins at the plasma membrane and (2) since the fluorogen does not fluoresce when it is not bound by SCA, we are able to completely eliminate background fluorescence when imaging in other channels. Although developed in yeast, this technology had surprisingly not been used to detect localization in this model system until our recent work looking at the cell surface residency of an ectopically expressed mammalian membrane protein, Kir2.1. To make this technology readily available to the cell biology community, we have first optimized the SCA sequence for expression in yeast, and then created a series of SCA-tagging constructs and organelle markers to be used as a tools for the cell biology research community. These tools will allow scientists to quantitatively analyze protein dynamics, minimize the effects of background, observe multiple fluorescent tags simultaneously in a single cell, and selectively illuminate the cell surface population of a tagged protein. This latter point is critical for quantitatively studying endocytic rates of specific proteins and will be an invaluable tool to the yeast and cell biology community.

351 The biosynthetic basis of cell size control

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Although cells of a given type span a large range of sizes, most proteins and RNA are maintained at constant, size independent, concentrations. This ensures that biochemical reactions proceed independently of cell size. However, our recent work in budding yeast has shown that a key cell cycle regulator, the G1/S transcriptional inhibitor Whi5, is synthesized independent of cell size to produce a size-dependent cell cycle-entry rate. This raises two fundamental questions: (1) Are there additional genes whose synthesis is decoupled from cell volume? (2) If most gene expression is proportional to cell size, what molecular mechanism promotes cell-size-independent gene expression? To address these questions, we analyzed flow cytometry data collected using the yeast GFP-fusion library. We identified approximately 100 genes whose expression depends weakly on cell volume and validated a subset of these candidates using quantitative live-cell microscopy. Gene ontology analysis revealed that proteins with weak cell-size dependence are enriched for genes with roles in DNA-templated processes and membrane transport, suggesting that cells employ differential protein synthesis to coordinate protein requirements with the scaling properties of cellular structures. Size-independent synthesis of Whi5 protein is due to size-independent transcription, which we measured using single molecule RNA FISH. To understand the mechanisms that underlie size-independent gene expression, we used transcriptional reporters of non-scaling genes, including WHI5, and determined that cell-size-independent regulation of some genes is due to non-scaling transcription rates. Finally, we show that asymmetric partitioning of proteins at mitosis mediated by chromatin association plays an important role in cell size-dependent protein homeostasis. Taken together, our work demonstrates a functional role for differential size-dependency of protein synthesis and gives insights into the underlying molecular mechanism(s).

352 Spontaneous mutations that confer resistance to 2-deoxyglucose act through Hxk2 and Snf1 pathways to regulate gene expression and HXT endocytosis

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Yeast and fast growing human tumor cells share metabolic similarities in that both cells use fermentation of glucose for energy and are highly sensitive to the toxic glucose analog 2-deoxyglucose (2DG). To define the molecular pathways that mediate resistance to 2DG, we sought to identify spontaneous yeast mutations that conferred resistance to 2DG using whole genome sequencing. We identified missense alleles of the HXK2, REG1, GLC7 and SNF1 genes and demonstrate that they confer significant resistance to 2-deoxyglucose. All three missense alleles identified in HXK2 resulted in a significant reduction in catalytic activity while, surprisingly, two of the HXK2 alleles had no effect on regulation of invertase gene expression. Missense alleles affecting the Snf1 kinase pathway (REG1, GLC7 and SNF1) each had different capacities to affect the regulation of invertase expression. Our earlier studies demonstrate that 2DG promotes the endocytosis of glucose transporters and that resistance to 2DG can arise from retention of glucose transporters at the cell surface. Consistent with this mode for 2DG resistance, all but one of the seven novel missense alleles identified in this study altered hexose transporter endocytosis and increased plasma membrane occupancy of the Hxt3 protein. In addition to altered glucose transporter trafficking, expression of the DOG phosphatases has been associated with resistance to 2DG. Expression of both the DOG1 and DOG2 mRNA was elevated after treatment with 2DG. Deletion of the HXK2 and REG1 genes confers resistance to 2DG and causes increased expression of the DOG2 mRNA. Based on these observations we find that Snf1 kinase-mediated regulation of the endocytosis of the hexose transporters and regulation of DOG2 expression are both important mechanisms for resistance to 2DG. Importantly though, the dominant SNF1-G53R allele is able to confer additional 2DG resistance in cells that are genetically compromised in both the endocytosis and DOG2 pathways. Thus at least one more as-yet undefined mechanism for conferring resistance to this glucose analog remains to be discovered. In the future, it will be interesting to define the conservation of these mechanisms in a cancer cell model.

353 Crosstalk Between Transcription, Splicing and Chromatin.

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There is extensive evidence that in both metazoans and budding yeast the process of splicing occurs as soon as the intron is transcribed and before transcription termination, i.e. co-transcriptionally. As a result, RNA polymerase II elongation rate can influence splicing, and splicing can affect transcription elongation. More recently, links between splicing and chromatin modification have also become evident. We have investigated the interactions between these three important cellular processes in budding yeast. Using mutations in the large subunit of RNA polymerase II that alter transcription elongation rate, we obtained evidence that slow RNA polymerase II elongation increases both co-transcriptional splicing and splicing efficiency and that faster elongation reduces co-transcriptional splicing and splicing efficiency, indicating that splicing is more efficient when co-transcriptional. Moreover, we demonstrate that splicing accuracy is sensitive to transcription rate; altering RNA polymerase II elongation rate in either direction compromises splicing fidelity, especially for ribosomal protein gene transcripts.

We also analysed histone modifications following rapid degradation of individual splicing factors, finding that defects in specific stages of splicing differentially affect H3K4 and H3K36 tri-methylation. Based on our results, we propose that transcription and chromatin likely respond to signals from splicing fidelity checkpoints.

354 Use of Structural Determination and Functional Complementation in Yeast to Search for Inhibitors of the Enzyme Deoxyhypusine Synthase of Eukaryotic Organisms that Cause Neglected Diseases.

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The eukaryotic translation factor 5A (eIF5A) is a highly conserved translation elongation factor in eukaryotes, and it is essential for the viability of all organisms tested so far. eIF5A undergoes a specific post-translational modification called hypusination, which is required for its function in the cell. Hypusination occurs in two steps, involving two different enzymes (deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase), and considering the essentiality of hypusination and eIF5A, these enzymes are interesting targets for the development of inhibitors that may become new drugs. Thus, we proposed the search for structural differences between the DHPS enzyme (first step of hypusination) of pathogenic organisms and human enzyme that may be useful for the development of inhibitors more selective for the pathogens that cause neglected tropical diseases. DHPS is essential in all tested organisms, and it is a potential target for therapeutic interventions, including those targeting human eukaryotic pathogens that cause neglected diseases. In addition, significant differences have already been described between the human enzyme and some parasites, which may contribute to discovery of specific drugs for these organisms. For this purpose, the deoxyhypusine synthase sequences of the following pathogenic eukaryotic organisms were chosen for structural determination and search for inhibitors: Leishmania major, Plasmodium vivax, Paracoccidioides brasiliensis, Histoplasma capsulatum and Brugia malayi. Also, we used a synthetic biology approach to generate complementation assays in Saccharomyces cerevisiae for screening of new pathogenic DHPS inhibitors, which minimally inhibits human DHPS.

355 Wild Saccharomyces cerevisiae lipid accumulation in lignocellulosic hydrolysate

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Microbial oils are currently the most promising feedstock for sustainable biodiesel production. Yeast is attracting considerable attention, but its low productivity on cheap crude substrates, such as lignocellulose-enriched residues, still hinders the industrial application of such oils. The development of robust yeast strains with increased oil yield and resistance to the different inhibitors present in the substrates is therefore one of the most important steps to improve the feasibility of microbial biodiesel.

Bioprospecting of wild environments frequently yields microorganisms with high resistance to a wide range of environmental stresses. In this project, wild S. cerevisiae strains isolated from spontaneous cachaça fermentation vessels in Brazil were screened for their potential to produce biodiesel from lignocellulosic residues. The strains that displayed high resistance to common lignocellulosic inhibitors were further assessed for lipid production in wheat straw hydrolysate. The strains' lipid accumulation profiles were evaluated by measuring growth and fluorescence emission, after addition of lipid-specific fluorescent dye BODIPY. The effects of carbon-to-nitrogen ratio (C/N) and temperature on biomass production and lipid accumulation were also assessed, in order to define the optimal lipid accumulation-inducing conditions for each strain. The best performing strains were further characterized in wheat straw hydrolysate and the lipid profile and accumulation assessed using GC-MS and GC-FID, to identify and quantify the main fatty acids present in the lipids.

356 Phosphate starvation promotes longevity via activation of autophagy and the MVB pathway in Saccharomyces cerevisiae

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In the last decades, several nutritional regimes, most and foremost caloric restriction and nitrogen starvation, were demonstrated to prolong lifespan of most organisms studied. Despite extensive research in this field, the influence of other macronutrients on cellular viability remains unknown. Here, we demonstrate that the restriction of phosphate extends chronological lifespan of the baker's yeast Saccharomyces cerevisiae. Taking advantage of its evolutionary highly conserved physiology, its easily modifiable genome and the ability to study the whole lifespan of this model organism within only a few days, we directly compared known dietary regimes with phosphate starvation, the later showing the most prominent lifespan extension of all tested dietary modalities. Our results indicate that a functional interplay of autophagy, the multivesicular body (MVB) pathway and the vacuolar fusion machinery is required for phosphate-induced longevity. Ongoing transcriptomic analysis will be used to determine the precise downstream events by which the nutritional limitation of phosphate promotes longevity.

357 Two-step response to replication stress: a dual role of the replication checkpoint

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To maintain genome integrity, cells slow DNA replication upon replication stress. The DNA-damage checkpoint contributes to this slowdown, but its quantitative role is only partially understood. Using time-resolved profiling, we find that cells exposed to the dNTP-depleting drug hydroxyurea (HU) slow replication in two phases: immediately upon exposure, before cell-cycle gene expression is perturbed, and following ~20 minutes, concomitant with cell-cycle transcription arrest. Deletion of the checkpoint-sensor (mec1) or the checkpoint-kinase (rad53) moderated the initial slowdown but prevented continuous replication following the secondary transition. I will discuss the contribution of several checkpoint-dependent signaling events in to the two temporal responses, and will present some initial data explaining the event triggering transition between the two phases.

358 Yeast As A Model System To Get Insights Into The Toxicity And Resistance Mechanisms To The Metabolic Inhibitor 2-Deoxyglucose.

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Cancer cells display an altered metabolism with increased glycolysis and glucose uptake. Anti-cancer strategies targeting glycolysis through metabolic inhibitors have been considered. The glucose analog 2-deoxyglucose (2DG) is imported into cells and after phosphorylation becomes 2DG-6-phosphate, a toxic by-product that inhibits glycolysis. 2DG has other cellular effects and can induce resistance. Using yeast as a model, we performed an unbiased, mass-spectrometry-based approach to probe the cellular effects of 2DG on the proteome and study resistance mechanisms. We found that two 2DG-6-phosphate phosphatases, Dog1 and Dog2, were induced upon exposure to 2DG and participated in 2DG detoxication. 2DG induced Dog2 by activating several signaling pathways, such as the MAPK (the p38 ortholog Hog1)-based stress-responsive pathway, the unfolded protein response (UPR) triggered by 2DG-induced ER stress, and the MAPK (Slt2)-based cell wall integrity (CWI) pathway. Thus, 2DG-induced interference with cellular signaling rewired the expression of these endogenous phosphatases to promote 2DG resistance. Consequently, loss of the UPR or CWI pathways led to 2DG hypersensitivity. In contrast, DOG2 was transcriptionally repressed by glucose availability through the inhibition of the Snf1/AMPK pathway, and glucose-repression mutants were 2DG-resistant. The characterization and genome resequencing of spontaneous 2DG-resistant mutants revealed that DOG2 overexpression was a common strategy to achieve 2DG resistance. A human Dog2 homolog, HDHD1, also displays 2DG-6-phosphate phosphatase activity in vitro, and its overexpression conferred 2DG resistance in HeLa cells, suggesting potential interference with chemotherapies involving 2DG.

359 Interplay of phosphate transport and TOR through Pho84 during Candida albicans gastrointestinal commensalism

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Candida albicans is a commensal fungal pathogen which colonized in the human gut by adapting to various nutrients in the microenvironment. Pho84, a phosphate transceptor, is known to be crucial for maintenance of both phosphate homeostasis and TORC1 activity which is required for Candida albicans virulence. In a mouse model of commensalism, loss of pho84 decreased C. albicans commensalism in both single and competitive mix infection. Overexpression of GTR1 or GTR1-GTP which hyperactivate TORC1 activity moderately rescued the gastrointestinal colonization defect. Furthermore, the colonization defect of the pho84 null mutant was partially complemented through feeding mice with high Pi diet. Interestingly, during gut commensalism high Pi significantly restores GI tract colonization to the WT level when combined with overexpression of GTR1 or GTR1-GTP in the pho84 null mutant. Finally, in vivo studies demonstrate that the downstream commensalism-related genes expression were upregulated with either high Pi or TORC1 activation. In vitro Caco2 cell infection model confirms that Pho84 promotes C. albicans adhesion to the intestinal epithelial cells through both Pi transport and TOR. Thus, our findings unravel a mechanism by which Pho84 promotes gut commensalism through the interplay between phosphate transport and TOR activation.

360 SGD and the Alliance of Genome Resources

Stacia Engel, Edith Wong, Rob Nash, Felix Gondwe, Kevin MacPherson, Patrick Ng, Suzi Aleksander, Stuart Miyasato and J. Michael Cherry

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The yeast research community has long enjoyed the support provided by the Saccharomyces Genome Database (SGD), and has flourished because of its existence, making great breakthroughs and technological advances, and contributing countless key insights to the fields of genetics and genomics over the past decades. SGD has recently joined forces with five other model organism databases (MODs) - WormBase, FlyBase, the ZebraFish Information Network, the Rat Genome Database, and Mouse Genome Informatics - plus the Gene Ontology Consortium (GOC) to form the Alliance of Genome Resources (the Alliance; alliancegenome.org). The mission of the Alliance is to develop and maintain sustainable genome information resources that facilitate the use of diverse model organisms to understand the genetic and genomic bases of human biology, health, and disease. The Alliance website integrates expertly-curated information on model organisms and the functioning of cellular systems. The easy-to-use interface enables unified access to comparative genomics and genetics data, facilitating cross-species analyses. The site is undergoing rapid development as we work to harmonize various datatypes across the various organisms. Explore your favorite genes in the Alliance to find information regarding orthology sets, gene expression, gene function, mutant phenotypes, alleles, disease associations and more! The Alliance is supported by NIH NHGRI U24HG002223-19S1, NIH NHGRI U41HG001315 (SGD), NIH NHGRI P41HG002659 (ZFIN), NIH NHGRI U24HG002223 (WormBase), MRC-UK MR/L001020/1 (WormBase), NIH NHGRI U41HG000739 (FlyBase), NIH NHLBI HL64541 (RGD), NIH NHGRI HG000330 (MGD), and NIH NHGRI U41HG002273 (GOC, which also provides funding to WB, MGD, SGD).

361 The E3 Ligase Rtt101/Cul4 facilitates lesion tolerance during replication stress

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Cullin-RING-based Ligases (CRLs) are a family of conserved E3 ubiquitin ligase that are regulators of DNA replication and repair. In baker's yeast, the CUL4 homolog RTT101 forms a multi-protein complex with MMS1 and MMS22. The RTT101-MMS1-MMS22 complex promotes DNA replication fork progression through damaged DNA and natural pause sites. The deletion of RTT101-MMS1-MMS22 renders cells sensitive to exogenous as well as endogenous sources of replication stress. The rtt101 strain is highly sensitive to the Top1 poison camptothecin (CPT) and the alkylating agent methyl methanosulfonate (MMS). In addition, rtt101 yeast are sensitive to hydroxyurea (HU), a Ribonucleotide Reductase (RNR) inhibitor, as well as the persistent accumulation of ribonucleotide monophosphates (rNMPs) within the genome. The replicative polymerases incorporate rNMPs at high frequencies during DNA replication. The Pol Epsilon allele, pol2-M644G, increases the genomic rNMP load more than 10-fold. Usually genomic rNMPs are of transient nature and are within each cell cycle removed by the ribonucleotide excision repair (RER) pathway. We mimic RER deficiency by auxin-inducible depletion of RNase H2.

These model systems enable us to study the molecular mechanism of Rtt101 function during these very different types of replication stress. In our current working model, we suggest that RTT101-MMS1-MMS22 modulates central replisome components to allow fork progression and/or restart after fork stalling.

362 Metabolic Engineering Of Yarrowia lipolytica For Plastic Degradation.

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Yarrowia lipolytica is a well-studied, unconventional yeast, which has natural ability for assimilation atypical carbon sources such as alkanes, polyols or fatty acids. This dimorphic yeast produces large amounts of bio-surfactants, lipases, and organic acids. Furthermore, it is resistant for low pH, how osmotic pressure and possesses a GRAS status.

Polyethylene terephthalate (PET) is the most common plastic material, which production increases every year. World's plastic pollution problem grew into a huge size issue. Up to now, two enzymes cutinase and PETase able to degrade plastic material, such as PET, PCL (poly(ε-caprolactone)) or PBS (poly(butylene succinate)) have been identified.

In this study, we overexpressed two genes, cutinase from Fusarium solani pisi and PETase form Ideonella sakaiensis in Y. lipolytica. Here we combined a natural ability of yeast for lipase production with its metabolic engineering to increase plastic degradation process.

The proteins were secreted due to signal sequence of XPR2 (protease) from Y. lipolitica. We integrated these two genes into different target places in the Y. lipolytica genome using EasyCloneYALI method, which is based on CRISPR/Cas9 system.

The resulting strains showed higher esterases activity than native stains, Fusarium solani pisi and Ideonella sakaiensis. Supernatants from obtained strains were also tested on FMM medium with 0,1% PCL. The results showed, that enzymatic activity vary, and it depends on the integration locus in the genome.

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363 DNA polymerase Epsilon is ubiquitylated in response to DNA damage

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During genome duplication, it is crucial to maintain the integrity of the genetic material to prevent potentially disease-causing abberations. Both exogenous and endogenous factors can give rise to replication stress-inducing lesions . One major source of endogenous replication stress is ribonucleotide monophosphates (rNMPs), that become erroneously incorporated into the DNA during normal replication. This misincorporation of rNMPs into the genomic DNA is usually transient as the RNase H2 enzyme removes rNMPs from DNA by ribonucleotide excision repair (RER). When RER is defective, e.g. due to mutations or deletion of the RNase H2 enzyme, rNMPs persist and induce replication stress.

CRLs (Cullin-RING-based Ligases) are a family of conserved E3 ubiquitin ligase. In budding yeast, the CUL4 homolog Rtt101 forms a multi-protein complex with Mms1 and likely with Mms22. Although Rtt101 associates with the replisome, its relevant targets have, so far, remained elusive. Interestingly, Rtt101 becomes essential when rNMPs accumulate in the genome. To better understand the underlying mechanism we aimed to identify the direct ubiquitylation targets of Rtt101Mms1-Mms22 during rNMP accumulation induced replication stress. Using a proteomic-based ubiquitin profiling approach, we found that RTT101Mms1-Mms22 polyubiquitylates the second largest polymerase 2 subunit, Dpb2, at Lysine-419. We hypothesize that Dpb2 is a crucial target of the Rtt101 E3 ligase when high levels of genomic rNMPs are present to promote replisome re-modelling, repair, and replication fork restart.

364 Functional profiling of inter-genic and intronic non coding RNAs

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The Saccharomyces cerevisiae genome has undergone extensive intron loss during its evolutionary history, and the few remaining may be retained because of their impact on function in specific environmental conditions. We explored the possibility that new functional ncRNAs are embedded within intronic sequences and are responsible to intron retention in yeast. We employed de novo RNA structure prediction tools to screen intronic sequences in 37 fungi, and we identified and validated 19 novel intronic RNAs via RT-PCR. We deleted the novel intronic RNA structure within the GLC7 intron and showed that this region, rather than the intron itself, is responsible for the cell's ability to respond to salt stress. RNA-seq analysis confirmed that introns in ribosomal protein genes are more highly expressed when they contain predicted RNA structures. Overall, these data support the notion that some introns may have been maintained in the genome because they harbor important RNA structures.

Our lab has also been involved in the construction of a barcoded ncRNA deletion collection in S. cerevisiae (ca. 450 mutants; Ref 2), and we are currently creating phenotypic and genetic interaction maps of ncRNAs in S. cerevisiae by exploiting (i) fitness analysis of double KO mutants generated using Synthetic Genetic Array (SGA) and (ii) screening the double KO library in stress conditions. Data on large-scale functional analysis (i.e. bar-seq and transcriptome data) and synthetic genetic interactions for the ncRNA collection will be presented: to date 27 ncRNAs have been used as query strains for SGA and several gene interactions have been discovered showing either loss or gain in fitness, such as for example ΔCUT084-ΔSUT083 or ΔSNR17-ΔCUT150.

365 Caffeine stabilizes fission yeast Wee1 in a Rad24-dependent manner but attenuates its expression under genotoxic conditions

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The ability to delay cell cycle progression in response to DNA damage or environmental stress is crucial for maintaining cellular viability. Caffeine has generated much interest due to its ability to override the DNA damage and replication checkpoints. Previously the inhibition of Schizosaccharomyces pombe (S. pombe) Rad3 and its functional homologues were proposed to be the target of caffeine's inhibitory activity. Recent findings indicate that the Target of Rapamycin Complex 1 (TORC1) is the preferred target of caffeine. Effective Cdc2 inhibition requires both the activation of the Wee1 kinase and inhibition of the Cdc25 phosphatase. The TORC1, DNA damage and environmental stress response pathways all converge on Cdc25 and Wee1. We previously demonstrated that caffeine overrides DNA damage checkpoints by modulating Cdc25 stability in S. pombe. The effect of caffeine on cell cycle progression under normal growth conditions resembles that of TORC1 inhibition. Caffeine also activates the Sty1 regulated environmental stress response. Caffeine may thus modulate cell cycle progression and checkpoints, by affecting multiple signalling pathways that regulate Cdc25 and Wee1 levels, localisation and activity. We have observed, that caffeine stabilizes both Cdc25 and Wee1. The stabilising effect of caffeine and genotoxic agents on Wee1 was dependent on the Rad24 chaperone that binds to both Cdc25 and Wee1. Caffeine inhibited the accumulation of Wee1 in response to DNA damage. Caffeine therefore, modulates cell cycle progression through increased Cdc25 activity and Wee1 repression under genotoxic conditions. TORC1 inhibition was sufficient to override checkpoint signalling, suggesting caffeine enhances sensitivity to genotoxins by inhibiting this signalling complex.

366 Improvement of Fermentative Profile of Saccharomyces eubayanus Using an Adaptive Laboratory Evolution Strategy

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The Lager beer yeast S. pastorianus is a hybrid between S. cerevisiae and the cryotolerant yeast S. eubayanus. The latest was recently found in Patagonia, however only a limited number of studies have evaluated its potential for beer production. Recently, our group isolated hundreds of S. eubayanus strains from west Patagonia (Chilean territory), showing a broad range of fermentative capacities and low ethanol production. To improve their fermentative capacity and ethanol tolerance, we adopted an Adaptive Laboratory Evolution strategy. Ten genetically identical groups combining 30 different strains from different isolation localities (Villarrica, Puyehue and Coyhaique) were incubated during 260 generations in a restrictive ethanol containing media (9% v/v). After 60 generations a sustained increase on ethanol tolerance was detected, showing a maximum fitness near 260 generations. Whole-genome sequencing of three ethanol and glucose-evolved lines (control) showed that a single genetic background prevailed after 260 generations (248.1, Villarrica), while the control lines showed different backgrounds at the end of the experimental evolution assay. Moreover, an aneuploidy of the chromosome 9 in the individuals of ethanol-evolved line 2 was observed. Additionally, a total of 53 mutated genes were detected considering the three ethanol evolved lines. Additionally, the best resulting ethanol evolved strain showed a 143% increase in their fermentation capacity in beer wort at 12 °C (similar to commercial lager strains). This represents the first step to obtain genetically improved Chilean wild yeasts for produce novel beers with unique organoleptic characteristics. This research is funded by: iBio Iniciativa Científica Milenio-MINECON, FONDECYT 1180161 Grant and FONDECYT PostDoctoral 3190532 Grant.

367 Recognition of H3 K14 Acetylation and H2B SUMOylation controls RSC Complex recruitment to Chromatin in vivo

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Chromatin-remodelling complexes are conserved machineries that govern chromatin dynamics through processes such as nucleosome assembly, nucleosome editing, and chromatin access. The Remodeller of Structure of Chromatin (RSC) complex is the most abundant and essential chromatin remodeller in yeast, which can recognise and remodel nucleosomes displaying specific post translational modifications, thereby playing pivotal roles in transcription, sister chromatid cohesion and cell cycle progression1. Mechanistic and functional insights into how this complex slides nucleosomes to modify chromatin structure are limited.

To shed new light on this question we use Genetic Code Expansion to install unnatural amino acids into histones2 and study the RSC: Nucleosomal Core Particles (NCPs) interaction, both in vivo and in vitro. Using a photoactivatable crosslinker we mapped the footprint of interaction of its catalytic subunit, Sth1 onto the nucleosome. We identified the crosslink of Sth1 to H3 K14 acetylated nucleosomes. Together with the double bromodomain of Rsc4, which recognizes the same acetylation mark, RSC should therefore preferentially bind symmetrically acetylated nucleosomes. We also showed that RSC preferentially binds to NCPs harbouring SUMOylated H2B in vivo. To evaluate its affinity, we reconstructed the interaction with the RSC complex and reconstituted SUMOylated NCPs in vitro. Binding analysis using Bio-layer interferometry showed that RSC's affinity for SUMOylated NCPs is doubled as compared to wildtype NCPs. Collectively, our findings show that both H4 K14 Acetylation and H2B SUMOylation containing NCPs serve as vital recruiting marks for RSC and this could further better our understanding of key cellular processes such as gene activation.

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368 Using yeast to model human diseases: parasites to paralysis

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The unity of molecular and cell biology across the Eukaryotes makes yeasts favourable organisms with which to model both infectious and systemic diseases of humans.

In order to model infectious diseases, we have set up systems in which the infectious agent and the human host are modelled either within the same or separate yeast cells. For instance, many tropical diseases are caused by eukaryotic pathogens, including protozoa and nematode worms, with a very similar biochemistry to humans. This makes it difficult to identify agents that will kill the parasite but not the patient. The search for such drugs is also hampered by the fact that many of these parasites are difficult or impossible to culture in the laboratory. This is where yeast comes in. We have developed a robust, fully automated method to screen for potential anti-parasitic drugs. This system uses separate yeast strains whose growth is dependent on the expression of coding sequences specifying target enzymes from either the parasite or the human host. The yeast system thus permits multiple parasite targets to be screened in parallel and is also able to exclude compounds that do not discriminate between host and parasite enzymes.

We have also used yeast to model the interactions between pathogenic bacteria and their plant and animal hosts; in this case both pathogen and host are modelled by a single yeast construct. We have engineered yeast to inducibly synthesise unusual nucleotides that are involved bacterial pathogenesis. Interactions between these bacterial signalling molecules and the yeast cell can result in growth inhibition or death. We find that cdiGMP functions through a mechanism that must be compensated by ribonucleotide reductase activity or by functionally competent mitochondria. Synthesis of the human Mesh1 protein in yeast indicates that it may be required to protect human cells from the damaging effects of ppGpp during bacterial infection.

The forgoing experiments generated data on yeast purine nucleotide metabolism that could not be accurately predicted by existing models of yeast metabolism. Investigation of this deficiency led us to increase the representation of iron metabolism in the model and to discover that yeast has potential for modelling the cellular processes in involved in the onset and early progression of Parkinson’s disease. I shall discuss other examples of using yeast to model neurodegenerative diseases, including Friedreich’s Ataxia and motor neurone disease.

369 Adaptation to Osmostress by the Hog1 SAPK

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Exposure of cells to osmostress results in the activation of the Hog1/p38 family of stress-activated protein kinases (SAPKs). Activation of these highly conserved MAP kinases 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, in yeast there is a major downregulation of gene expression that is bypassed specifically in stress-responsive genes by the action of the Hog1 SAPK which acts in multiple stepts of mRNA biogenesis. In addition to regulate transcription, SAPKs control cell cycle progression. For instance, Hog1 modulates the G1/S transition by targeting core components of the cell cycle machinery such as CDK inhibitors as well as by regulating cell cycle gene expression. In addition, a novel checkpoint in S phase controlled by SAPKs is critical to coordinate transcription and replication allowing for full stress-responsive transcription during S phase without affecting DNA integrity. All together highlights the relevance of this signaling pathway in the control of several aspects of the cell physiology to maximize cell survival in the presence of stress.

370 Gaining New Insights in Yeast Cell-Cycle Regulation by Collection of Correlated Single Cell Data. A Multi Sequential mRNA FISH Approach

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The cell-cycle of genetically identical yeast cells is robust even though the individual biochemical reactions responsible for the cell-cycle regulation are stochastic processes.

Progression of cells through the cell-cycle is a very complex, highly regulated process. Main players for the orchestration through the cell-cycle are cyclins, cyclin dependent kinases, inhibitors and transcription factors. Most of them show oscillating gene expression, typically in low mRNA numbers. To get absolute transcript numbers of the expressed genes in single cells we perform multi sequential single molecule fluorescent in situ hybridization (MuSeq-FISH and smFISH) on a series of 21 target mRNAs, which are labeled successively in sets of three. Besides the experimental optimization, we are developing an image analysis workflow. We are aiming for correlated transcript numbers of the genes in single cells.

The project will result in a very new quality of cell-cycle phase resolved single cell data, to be used for refined cell-cycle modelling.

371 Role of calmodulin in turning a metacaspase 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. In this work, we show that the yeast metacaspase Mca1 displays dual activities: canonical metacaspase protease activity that triggers cell death and co-chaperone-like activity that retards aging. We report that these dual activities are regulated by calmodulin binding to the N-terminal domain of Mca1 to favor co-chaperone over protease activity, which in turn prevents apoptosis and assures longevity. Longevity-promoting effects of Mca1 required the Hsp40 chaperone Sis1, which we show is necessary for Mca1 recruitment to protein aggregates and Mca1-dependent disaggregation of misfolded proteins. Activated metacaspase protease activity, in contrast, resulted in Sis1 fragmentation both in vitro and in vivo, further pointing to a dual, and antagonistic, activity of Mca1, as an auxiliary positive factor in Sis1-dependent protein quality control/longevity assurance and as a metacaspase triggering Sis1 fragmentation and apoptosis.

372 A stress granule formation pathway identified from yeast imaging-based phenomic screens

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Stress granules (SGs) are non-membranous organelles facilitating stress responses and linking the pathology of age-related diseases. Great efforts have been made in investigating the structure, assembly, and dynamics of SGs, but little is known about signaling pathways controlling SG formation. By developing and combing 2 yeast high-content imaging-based phenomic screens, we identified a signaling pathway regulating SG formation through the phase separation of a cytosolic Lsm (Like Sm) protein. We demonstrate that under glucose starvation stress TORC1/2 function in an early step of SG formation, leading to a decreased function of the sphingolipid synthesis pathway. This further down-regulates an ubiquitin proteasome component, which in turns decreases the phase separation of the Lsm protein. We further show that the Lsm foci function as structural foundations or scaffolds that promote SG formation. The signaling pathway identified in this work together with its conserved components provides us with vital clues for understanding the mechanisms underlying SG formation and potentially SG-associated human diseases.

373 Quantitative proteomics reveals genome scale responses of S.cerevisiae to reduced metal availability and predicts novel gene functions

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Living organisms are built primarily of 6 non-metals (C, H, O, N, P, S), Na, K, Ca, Cl, Mg and ≈5 other trace elements (Zn, Cu, Fe, Mn, Mo). The concentrations of non-metals inside cells are much higher than that of others, however, even elements present in minute quantities influence cellular physiology. Although some functions of these trace elements (e.g. the role of transition metals in catalyzing enzymatic reactions) are well known, there remains a lot to learn from studies that systematically investigate the connections between metal ions and each component of a S.cerevisiae cell . We use well established, systems scale, molecular biology and biochemistry tools to investigate interactions between metal ions and multiple functional layers of S.cerevisiae cells. This poster summarizes the results of a quantitative proteomics experiment which revealed that the depletion of each metal ion results in a unique differential expression (DE) signature. The DE signatures are significantly enriched for known metal related biological functions and predict novel functional associations, both for well known and uncharacterized ORFs.

374 The Different Roles of Cytosolic Hsp70s in Proteostasis and Lifespan Regulation

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The four cytosolic 70 kDa heat shock proteins (Hsp70s) in yeast, Ssa1-4, are key molecular chaperones that ensure cellular fitness through regulation of protein homeostasis. The Ssas have many of the same functions, yet a lack of Ssa1/2 causes a reduction in cellular fitness and a shortening of replicative life span, even though Ssa3 and 4 are present. We set out to answer the questions that arises from this fact – what are the essential functions of the Ssa chaperones that ensure cellular fitness and longevity, and are all Ssas capable to carry out these functions? By overproducing Ssa4 in cells lacking Ssa1/2, we found that Ssa4 can rescue growth, reduce the formation of protein aggregates during non-stress conditions, promote the formation of inclusion bodies after heat stress and increase the rate of degradation of misfolded proteins. However, overproducing Ssa4 in the absence of Ssa1/2 did not fully restore the clearance of protein aggregates or inclusion bodies after stress, nor did it restore the recruitment of the disaggregase Hsp104 to aggregated proteins. The recruitment of Hsp104 to protein aggregates could be restored by replacing the nucleotide-binding domain of Ssa4 with the same domain from Ssa1, which points towards functional differences between the NBD of Ssa4 and Ssa1. Despite this functional “defect”, Ssa4 was able to restore the short lifespan of cells lacking Ssa1/2 to levels comparable to the wild type, which leads to our conclusion that Hsp70/Hsp104-dependent clearance of protein aggregates is not needed to ensure a normal lifespan.

375 Cryo-EM reveals the immature and mature dengue VLP structures

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Dengue virus (DENV) belongs to Flaviviridae family and has four serotypes. DENV surface proteins are mainly composed of envelope (E), membrane (M) and capsid (C) proteins. In the process of virus replication, newly synthesized DENVs are assembled as immature particles which have pr-M protein. The furin then cleaves away pr so that the mature DENV contains only M protein and is infectious. However, the furin cleavage is thought to be inefficient. It is believed that a completely immature DENV lacks the ability to infect cell, but cells infected with dengue virus release a high proportion of immature virions and the immature DENV could enter to the cells through antibody dependent enhancement leading to following severe pathogenesis. Our previous study (Shen et al., 2018, eLife) showed that mature dengue viral like particle could neutralize four serotypes and was therefore suggested to be a safe vaccine candidate. Here, we would like to show the cryo-EM structures of dengue VLP in immature and mature forms using cryo-EM. The cryo-EM structures showed that immature VLP had protrusions on the surface while mature VLP had smooth structure. Surface proteins lied parallelly on the surface and followed T=1 lattice arrangement in both cases. The structures could tell us the surface protein rearrangement and epitope exposure changes during maturation process.

376 DNA double-strand break repair mechanisms and resection within a CAG/CTG microsatellites

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Neurodegenerative diseases are today the third cause of death in France behind cardiovascular diseases and cancer and no cure is available for them. Several neurodegenerative disorders are the result of a large expansion of a CAG or CTG microsatellite, such as myotonic dystrophy type 1 (DM1) or Hungtinton disease. Such diseases are transmitted from parents to children, with an expansion of the microsatellite at each generation. The number of repeats and the severity of the disease are correlated. Some cancers (HNPCC and colorectal cancers) and fragile sites are also linked to microsatellite instability.

In an earlier work, a TALE-Nuclease (TALEN) was specifically designed to generate a single DSB within the CAG/CTG repeats of a DM1 patient integrated in the yeast Saccharomyces cerevisiae. One arm of the TALEN recognizes the CAG/CTG repeats, while the other arm only recognizes the 3' end of the repeat, along with the adjacent non-repeated sequence, therefore inducing the DSB at the frontier between repeated and non-repeated DNA. We therefore have a unique model allowing us to compare, at the same time and in the exact same experimental conditions, resection of a repeated and structured DNA sequence to a non-repeated one. We showed that inducing a double-strand break (DSB) within CAG/CTG repeats induced a high frequency of repeat contraction in yeast. We also showed that resection of the repeated end was less efficient than resection of the non-repeated DSB end. Establishing the same results in human cells could be a possible approach to treatments by gene therapy.

In the present project, we aim at better understanding the mechanisms involved in DSB repair that occurs within a CTG microsatellite. Several genes of the repair machinery were chosen for their role in DSB resection and repair (MRE11, SAE2, EXO1, SGS1, DNA2). We show that the Mre11 nuclease activity and Sae2 activity are crucial for an efficient repair of the DSB. If the nuclease activity of Mre11 is hindered (mre11D56N, mre11H125N) or if SAE2 is knocked out, DSB repair is very inefficient and goes with a high mortality rate, resection of the DSB is slowed down to the point of being almost completely lost within the repeats. Moreover, while the repeats are poorly resected, this is not the case for the non-repeated DSB end. Later resection steps are activated by Exo1 and Dna2 (in complex with the helicase Sgs1). In an exo1Δ mutant the cells are still able to process the DSB but with lower efficiency than wild type cells. In order to better understand the role played by SGS1 and the essential gene DNA2 "Anchor Away" strains were also designed and are being studied.

377 Biotransformation of the Pectin Degradation Product D-Galacturonic Acid to L-Galactonate in Saccharomyces cerevisiae

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As one of the most abundant polysaccharides in nature and a waste product of sugar industry, pectin ranks as one of the major potential second-generation feedstocks (non-food plant material) for biotechnological production of different compounds, such as fuels, fine chemicals and additives for cosmetic and nutraceutical industry. Pectin mainly consists of α-1,4-glycosidically linked D-galacturonic acid units (D-GalA), which is naturally not metabolized by the prominent biotechnological production host Saccharomyces cerevisiae. We aim to engineer this yeast for biotransformation of D-GalA to L-galactonate (L-GalOA), which has potential applications as chelator, moisturizer, pH-stabilizer and leaving agent in food and cosmetic industry. This requires the expression of heterologous D-GalA transporters and reductases (Benz et al., 2014), but also extensive interventions into the central carbon metabolism of the host cell. Previous attempts to construct D-GalA utilizing S. cerevisiae strains have revealed that the higher oxidation state of D-GalA (compared to sugars) is one of the challenges for its funneling into the endogenous metabolism of yeast (Biz et al., 2016), since surplus reduction equivalents are required. These can be derived from the fermentation of sugars present in the pectin biomass, mainly glucose, galactose and arabinose by blocking the ethanol formation. In this way, a nearly complete valorization of the pectin feedstock, which is currently underused, can be achieved.

378 Effects of Temperature and pH on Saccharomyces paradoxus Killer Yeasts

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Saccharomyces paradoxus is the closest known relative of Saccharomyces cerevisiae. While S. cerevisiae is a domesticated and well established model organism in research, S. paradoxus thrives in wild natural habitats and shows considerably more genetic diversity within the species than S. cerevisiae. Both species share the capability of hosting Totiviridae dsRNA viruses often related to killer phenotype. It has been demonstrated that at least some S. paradoxus dsRNA viruses are compatible with S. cerevisiae cells and can provide the killer phenotype to any species. However, the distribution of different types of viral dsRNA naturally maintained in either species appears to be species-specific.

Yeast dsRNA-based killer system consists of L-A and M type dsRNA viruses. L-A virus is responsible for the production of viral proteins required for maintenance of L-A and M dsRNAs, therefore it is crucial for the survival of both viruses. Functional M dsRNA encodes for killer toxin and confers self-immunity. Preservation of L-A and M viruses provides evident profit for host cell by enabling the production of a specific secreted killer toxin which impedes survival of toxin-sensitive competitor cells.

In this study, we report novel S.paradoxus killer strains isolated from spontaneous fermentations of black chokeberry (CKB-17-33) and serviceberries (AML-15-66). Both strains harbour two types of dsRNA viruses of Totiviridae family, namely, L-A (approx. 4,6 kb) and M (ranging from 1,3 to 1,6 kb), responsible for the killing phenotype. S. paradoxus K66 and K33 toxins were isolated and the essential parameters of protein activity were investigated. We observed that AML-15-66 and CKB-17-33 exhibit killing activity against non-killer and different types of killer virus-possessing strains at pH ranging from 3.6 to 5.6. Importance of temperature and pH of the growth medium for the killer phenotype stability was investigated.

379 Adaptive Evolution of Saccharomyces cerevisiae PE-2 to Treatments of High Ethanol Content

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One important trait sought in industrial yeasts is their tolerance to high ethanol titers present at the end of very high gravity fermentations, such as for the production of ethanol as biofuel. Even a slight increase in ethanol tolerance by fermenting yeasts might have a huge impact on large-scale productivity by bioethanol powerplants. Here we used adaptive laboratory evolution as a valuable tool to select for ethanol tolerant yeast strains. Basically, we conducted an ethanol survival experiment in which four haploid Saccharomyces cerevisiae PE-2 populations were submitted to harsh ethanol treatments for two hours at 32 °C, followed by a recovery period in an ethanol-free medium (2-4 days). Such cycles of shock/recovery were reinterred with increasing ethanol content. The ethanol treatment started from an initial shock of 19% (v/v) ethanol (during which most of the cells died) and progressed through about 70-80 cycles of shock/recovery, until the four populations were well adapted to shocks of 28-30% (v/v) ethanol. Competition assays between the evolved populations and the ancestor show a clear pattern of antagonistic pleiotropy, in which the evolved strains achieve higher fitness than the progenitor to tolerate ethanol shocks, however they are largely outcompeted by the ancestor under normal growth conditions and even during propagation on liquid medium containing 8% (v/v) ethanol. Whole genome sequencing (Illumina MiSeq platform) recovered 67 point mutations across the four final populations. Functional analysis of the affected genes suggests a prominent role of trehalose accumulation and inhibition of the RAS/PKA pathway in improving survival rates to ethanol shocks. Molecular genetic analysis of key mutations found during whole genome sequencing are currently underway and will allow a fine understanding of the evolution process.

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380 Metabolic Engineering of Saccharomyces cerevisiae for the Production of Vitamin B12.

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Vitamin B12 is an essential cofactor for two enzymatic reactions in the human metabolism: the production of methionine and methylmalonyl-CoA . The human body requires only around 2,4 µg/day to provide a sufficient amount of the cofactor for the enzymes methionine synthase and methylmalonyl-coA-mutase (Food and Nutrition Board 1998). Despite the small amount, these reactions are crucial for a healthy human metabolism as vitamin B12 deficiency can lead to hematological as well as neurological disorders (Shipton and Thachil 2015). Humans require an external source of B12, as only microorganisms can produce vitamin B12 de novo (Fang, Kang, and Zhang 2017). To date mainly the bacteria Propionibacterium shermanii, Pseudomonas denitrificans and Sinorhizobium meliloti are deployed for industrial production processes. However, the cultivation of such bears several limitations, as time intensive fermentations in expensive media under complex conditions hamper the production efficiency (Martens et al. 2002). Furthermore, these organisms are not accessible through established biotechnological and genetical tools, which are widely understood as the key instruments towards high yield production hosts. Hence, a completely new approach is required to maximize the potential of microbes for vitamin B12 production. Due to the high amount of tools for metabolic engineering, as well as low fermentation costs, high robustness and a big spectrum of usable analytical tools, Saccharomyces cerevisiae serves as a suitable host for heterologous vitamin B12 production (Borodina and Nielsen 2014; Gottardi et al. 2017). For this reason, we aim to establish enzymes from organisms that can produce vitamin B12 de novo or using a salvage pathway to stepwise introduce the whole de novo producing pathway into the genome of S. cerevisiae. Further we develop a growth based screening system in S. cerevisiae for a bio based determination of vitamin B12 production.

Once vitamin B12 is produced in yeast, the strains and associated tools can not only be used for further strain development, but the purified product or inactivated cells themselves can serve as a nutritional food additive to increase dietary vitamin B12 intake as it is done already in form of nutritional yeast.

381 Acetoacetyl-CoA Pathway: Improving 1-butanol Production By Pathway Compartmentalization In Yeast And Extending The Range Of Products.

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The increasing demand for oleochemicals and the need of more sustainable production processes have boosted the interest on biological systems as producing platforms for medium and long chain molecules. In biotechnology, the chain elongation required for the synthesis of such molecules has historically been achieved by engineering the fatty acid biosynthesis pathway (Clomburg et al. 2017; Dellomonaco et al. 2011). However, chain elongation through the reversal of the β-oxidation (Acetoacetyl-CoA pathway) is also possible and is more energetically favourable (Dellomonaco et al. 2011; Clomburg et al. 2017), since the intermediates are not activated with ACP, at the cost of one ATP, but with Coenzyme A. Such elongation mechanism is already present in nature, where Clostridia produce n-butanol starting from Acetyl-CoA. Schadeweg and Boles (2016) achieved the highest 1-butanol titer in S. cerevisiae (859 mg/L) by expressing a modified clostridial pathway in the cytosol. However, competing reactions or a suboptimal environment could be limiting the production yield. A promising solution to this could be the compartmentalization of the pathway in other organelles. This strategy was successful for the production of other biofuels in yeast (Hammer and Avalos 2017). Apart from 1-butanol, reversal of the β-oxidation have been successful for the production of medium chain alcohols and acids like hexanol, hexanoic acid (Clomburg et al. 2017; Cheon et al. 2014; Mehrer et al. 2018) or octanol (Mehrer et al. 2018). However, the enzymes and intermediates from the reverse β-oxidation can be used for the synthesis of other interesting compounds besides linear molecules, as demonstrated recently for the synthesis of cannabinoids in yeast (Luo et al. 2019).

Here, we study the effect of pathway compartmentalization in the production of 1-butanol. For this, novel signalling tags aiming those organelles were fused to enzymes involved in the pathway and the metabolism of S. cerevisiae was engineered to push the carbon flux towards these organelles. Furthermore, we also study the production of interesting chemicals from intermediates of the pathway.

382 Production of soluble secreted mCherry in Pichia pastoris and its applications

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The methylotrophic yeast Komagataella phaffii (Pichia pastoris) is among the most widely used expression hosts for recombinant protein production. Taking advantage of its strong methanol inducible promoters (e.g. pAOX1) and its high secretion capacity, high product titers can be reached and the system is constantly being engineered to further enhance production efficiency.

The fluorescent protein mCherry is well described for its high pH and photo stability (Exc.: 587nm, Emm. 640nm) and commonly applied to visualize intracellular compartments or, fused to proteins, to trace their fate within the cell. Rather rarely it is used for the secretion into the extracellular space.

Aiming for a rapid and efficient method to quantify the influence of engineering strategies on secreted protein, we designed an expression cassette for secretion of mCherry protein in Pichia pastoris. The protein was C-terminally fused to the alpha-mating factor leader sequence of S. cerevisiae and is expressed under the control of the constitutive GAP promoter.

We developed a fast detection assay for the accurate and reproducible quantification of mCherry in the culture supernatant using simple fluorescence measurement in a 96-well format. Native PAGE and mass spectrometry analyses confirmed the production of the correctly folded protein.

With this rapid quantification method, mCherry can be used as a model protein for the fast and easy assessment of the success of new engineering strategies. This knowledge can be used to identify promising candidate strains for the production of more complex and difficult to analyze proteins.

383 Non-AUG Translation Initiation Results in Localization of Proteins Involved in RNA Metabolism to the Mitochondria

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Non-AUG translation initiation can lead to the production of extended protein isoforms that localize to the mitochondria. Recent evidence from our lab identified a number of proteins involved in RNA processing that are predicted to gain mitochondrial targeting signals and localize to mitochondria based on ribosome profiling data and confirmed in some instances. Included in this group are proteins known to function in RNA surveillance and turnover, such as Mtr4, Lsm1, Lsm4, and Lsm6, which were demonstrated to be in the mitochondrial fraction. LSM1 and MTR4 strains lacking the N-terminal extensions, expressing only from the canonical AUG, localize in the cytosolic fraction and not in the mitochondria, indicating that the N-terminal extension is required for mitochondrial localization. Cells lacking LSM1 or LSM6 have a petite phenotype, indicating impairment of mitochondrial function. Although other Lsm proteins do not have apparent mitochondrial targeting signals, Lsm3 and Lsm7 also localize to mitochondria. Co-immunoprecipitation experiments using dual-tagged strains show interactions between Lsm1 and Lsm7, Lsm4 and Lsm7, and Lsm6 and Lsm7. Lsm2, Lsm5, and Lsm8 have not been identified in the mitochondrial fraction. Currently we are investigating the function(s) of the Lsm and Mtr4 proteins in the mitochondria.

384 Discovering Age-Specific Post-translational Modifications (PTMs) of Budding Yeast Centrosomes.

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The spindle pole body (SPB) is the microtubule-organizing center in yeast cells. Each cell division, the SPB duplicates to form the bipolar mitotic spindle that segregates the chromosomes. The duplication of the SPB is mainly conservative and generates an old and a new SPB. Intriguingly, the two SPBs segregate in an age-specific manner in budding yeast; the mother cell retains the new SPB while the bud inherits the old SPB. However, how the cells identify old- and new-SPBs is unclear. One attractive hypothesis is that, since old and new SPBs are made during different stages of the life of the cell, they acquire distinct post-translational modifications (PTMs) that are recognized by the spindle positioning machinery. In support of this hypothesis, a recent study has identified three enzymes (the kinases Swe1 and Kin3 and the acetyltransferase NuA4) that control the age-specification of Nud1, a component of the SPB outer plaque.

We aim to identify age-specific PTMs of budding yeast SPBs. To this end, we use a technique named recombination induced tag exchange (RITE), which is a ‘living pulse-chase’ that allows the genetic switch between two epitope tags. With this technique, we could differentially label old- and new-SPB proteins in living yeast and further separate them by tandem affinity purification. As proof of principle, we used this method to purify old- and new-Spc110, which is the SPB receptor of nuclear microtubules. Then, we analyzed the purified proteins by mass spectrometry and identified 14 out of 16 known phosphorylation sites. Remarkably, we found two potential age-specific phosphorylation sites. Our current goals are to validate these age-specific phosphorylation sites, to explore their function and to expand our analyses to other SPB proteins.

385 Uncovering Genes Involved in Cell-Cell Fusion During the Mating of Yeast.

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Cell-cell fusion is a fundamental mechanism in eukaryotic cell biology. During fertilization, mating gametes fuse their plasma membranes (PMs) to generate a diploid zygote, the first step in creating new life. Surprisingly, little is known about how proteins mediate gamete cell fusion in vertebrates and fungi. To investigate PM fusion during fertilization, we use the budding yeast Saccharomyces cerevisiae as a simple eukaryotic model system. By utilizing a split-GFP bimolecular complementation-based flow cytometry assay, we have completed a quantitative cell fusion screen and identified 120 genes whose deletion results in a cell fusion defect. Interestingly, our findings reveal that the deletion of almost all components of the yeast V-ATPase and V-ATPase-associated factors leads to a defect in the last stages of cell fusion during yeast mating. The V-ATPase is important for acidification of the vacuole and other endocytic compartments. Therefore, the complete knockout or inhibition of the complex leads to defects in endocytosis, signaling, intracellular trafficking, and other housekeeping functions. These findings raise the question of the direct or indirect role of the V-ATPase during cell fusion, which forms the focus of our study. By employing confocal and cryo-electron microscopy, we anticipate to ascertain and classify the fusion defects presented by the V-ATPase mutants and all other fusion defective candidates. Thereafter, functional and mechanistic characterization of these genes to uncover their role during cell fusion in fertilization will be performed. This will shed light on understanding of sexual reproduction of organisms and the creation of life.

386 Role of Eisosomal Proteins in Yeast Mating.

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Cell-cell fusion is a fundamental process in eukaryotic sexual reproduction and development. Cell fusion proteins, i.e. fusogens, mediate membrane fusion by dehydrating the membrane polar head groups, promoting a hemifusion stalk formation, and opening and expanding a fusion pore. Despite the diversity of organisms and cell types that utilize cell fusion, the bona fide fusogens and the mechanism underlying this process remain elusive. Yeast mating provides a genetically amenable model organism for understanding late cell fusion events. In an effort to identify the yeast fusogenic machinery and the underlying molecular mechanism, we completed a proteomics analysis of plasma membrane pheromone up-regulated proteins revealing approximately 20 proteins with unknown functions in yeast mating. Pun1p, a protein previously implicated to localize to the plasma membrane compartment of Can1(MCC)/eisosomes, localizes to the tip of the 'Shmoo' and to the Fertilization Synapse generated immediately before plasma membrane fusion during yeast mating. On the other hand, its putative paralogs (SUR7, FMP45 and YNL194C) together with other major eisosomal proteins are downregulated upon pheromone treatment. Pun1p shares the conserved Claudin-like cysteine motif GLWxxC(8-10 aa)C at its first extracellular loop, similar to Fig1p, a known protein involved in the plasma membrane fusion step of mating. Deletion of PUN1 and its putative paralogs presents mild fusion defects. This is consistent with the observed down-regulation of eisosomal proteins. To further understand the role of Pun1p in yeast mating, we are currently performing epistatic analysis of pun1 and known genes involved in mating. In addition, we aim to carry out affinity pull-down experiments to identify Pun1p interacting partners with possible roles in fusion. Ultimately, these efforts may provide clues to the role of eisosomes in yeast mating, offering new insights into yeast cell-fusion events.

387 Identifying Novel Fusion Proteins in S. cerevisiae Mating Using Suppression and Sufficiency Criteria.

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Membrane fusion underlies a variety of important biological processes such as fertilisation. However, biological membranes do not spontaneously fuse. As such, biological membrane fusion events have been found to be mediated by specialised proteins known as fusogens. Many fusogens remain elusive. Our goal is to identify putative fusogen(s) and other proteins responsible for membrane fusion during mating of S. cerevisiae.

To this end, we aim to perform two systematic overexpression screens utilising a plasmid based genomic library. The first aims to identify genes involved in the yeast mating pathway on the basis of suppression of mating defective mutants when the gene is overexpressed. The second aims to discover proteins which are by themselves sufficiently able to facilitate membrane merger between yeast cells when overexpressed. As a proof of principle, we aim to heterologously express a characterised fusogen from C. elegans, epithelial fusion factor (EFF-1) to demonstrate sufficient fusogen-mediated fusion between yeast cells.

Initial expression of EFF1-mCherry in yeast was observed to be vacuolar, however replacement of the EFF-1 signal peptide with the yeast Kar2 signal sequence (Kar2SP-EFF1) led to membrane localization. Further suppression experiments will determine whether Kar2SP-EFF1 is functional in vivo. The identification of novel molecular determinants of membrane fusion will provide greater insight into how cell-cell fusion behaves from a mechanistic and evolutionary standpoint.

388 Unexpected Evolutionary Trajectories Of Lactose And Cellobiose-Assimilation Genes In The Kluyveromyces Genus

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Lactose utilisation is considered a defining feature of the yeasts Kluyveromyces lactis and Kluyveromyces marxianus and is a trait absent from most other yeasts. Even within the Kluyveromyces genus, however, it is variable, and some species, notably K. lactis var. drosophilarum and K. dobzhanskii, are unable to assimilate lactose. Two genes, LAC12 and LAC4, encode a permease and beta-galactosidase, respectively that allow lactose utilisation, with a variant of Lac12 responsible for enhanced uptake in a specific K. marxianus dairy lineage. Our studies of the evolution of the LAC genes established that LAC12 and LAC4 are ancestral in the Kluyveromyces genus but were lost in the evolutionary branch leading to K. lactis and K. dobzhanskii. In a clear demonstration of a domestication event, however, a 15kb introgression from the dairy lineage of K. marxianus to K. lactis restored lactose utilisation to K. lactis var. lactis. Furthermore, we found that Lac12 is also an effective cellobiose transporter and, in conjunction with a beta-glucosidase, Cel2, enables K. marxianus grow on cellobiose. Other Kluyveromyces species have a dedicated cellobiose transporter, Cel1, which has been lost by K. marxianus, and this work led to identification of a novel fungal cellobiose utilisation system, encoded by the CEL1 and CEL2 genes. The LAC12-LAC4 and the CEL1-CEL2 systems are ancestral and we demonstrate how a fascinating pattern of gene loss, duplication, transfer and neofunctionalisation shaped sugar utilisation in the Kluyveromyces genus.

389 Exploring the Role and Mechanisms of BIK1 at the Mitotic Spindle

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Microtubules (MTs) are protein filaments that make up the mitotic spindle during chromosome segregation. Plus end-binding proteins (+TIP) regulate MT stability and facilitate interactions with other proteins in order for proper segregation to occur. One such +TIP, BIK1, exists in several distinct pools within the cell: A mobile cytoplasmic pool at astral microtubules emanating from the spindle pole body (SPB) and a stable nuclear pool localised close to the SPB and kinetochores. The cytoplasmic pool of BIK1 has been extensively described in it's role during spindle positioning. By contrast, the function, dynamics and regulation of nuclear BIK1 remains unclear.

We recently identified BIK1 in a screen searching for proteins with age-dependent subcellular localisation. We found that BIK1 is loaded at kinetochores early in the cell cycle and remains there until the metaphase to anaphase transition. As cells enter anaphase pre-existing BIK1 relocalizes from the kinetochores to the interpolar microtubules and finally to the spindle midzone. By contrast, the astral pool of BIK1 is made de novo. To explore the nuclear function of BIK1 we fused a strong nuclear export signal to a GFP-tagged variant (BIK1-NES). In the BIK1-NES mutant strain a subset of cells display segregation defects with delayed spindle elongation. We are now investigating the molecular pathways regulating nuclear BIK1 function using genetic and biochemical approaches

390 A genome-wide screen for genes required for yeast to resist visible light pinpoints protein kinase A as a major determinant of light resistance

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To provide mechanistic insight into cellular responses to visible light in the range 400 to 700 nm, we introduce a methodology for high-throughput screening of gene-light interactions using the yeast gene deletion collection. Detecting gene-light interactions on yeast fitness is challenging because of a strong dependence between the cell density in a yeast colony and the growth during light exposure. Nevertheless, using a two-tiered validation procedure we identified 494 genes required for light resistance at physiologically relevant conditions using a semi-high throughput method and more than 20 genes also in individual assays. Light-sensitive mutants were involved in a wide array of functions including MAPK signaling, protein translation and protein modification (in particular diphthamide biosynthesis) and there was a high degree of overlap with genes required for oxidative stress resistance. On the contrary, genes required for mitochondrial functions were strongly depleted in the sensitive set pointing to mitochondria being responsible for light-induced oxidative stress. Classification of mutants based on the nucleocytoplasmic shuttling of the stress responsive transcription factor Msn2 as well as on glycogen accumulation, indicated enhanced protein kinase A activity to be a common denominator in several of the light-sensitive mutants and we confirmed that PKA repression was essential for cells to grow upon illumination. Taken together, our data links yeast light resistance to a pathway governing many metabolic and morphological outputs suggesting that light may have shaped the evolution of baker's yeast and other non-photosynthetic organisms.

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