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International Specialised Symposium on Yeasts ISSY25

Systems Biology of Yeasts – from Models to Applications

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Otamedia Oy, Espoo 2006

Preface

Dear Colleagues,

On behalf of the International Commission on Yeasts (ICY/IUMS) and the local organising committee, I am delighted to welcome you to Finland to the 25th International Specialised Symposium on Yeasts, ISSY25, this year dedicated to "Yeast systems biology – from models to applications".

The symposium has attracted a great number of participants. We quickly reached the maximum number of attendees that the Hanasaari Conference Centre can hold. We regret and sincerely apologise that we had to turn down some colleagues who wanted to join the meeting at a later stage. The 240 participants of the symposium come from 29 countries. The abstracts we have received are of excellent quality and many more of them deserved to be selected for oral presentations than we have time for. We anticipate high quality lectures and poster sessions with interesting and inspiring discussions during the meeting!

The interest which the ISSY25 meeting has received and the quality of the science to be presented shows the importance of systems biology and the significant role of yeast in this new field of biology. The position of yeasts, with *Saccharomyces cerevisiae* as the forerunner, as model organisms for fundamental science and as hosts in industrial production continues to be strong. The special issue of FEMS Yeast Research dedicated to this symposium will ensure that the results presented at Hanasaari Conference Centre will spread to a wider audience.

We thank the local organising committee members, the international scientific committee and our colleagues at VTT for their help. We are very grateful to our industrial and organisational sponsors for their financial contributions; without their support this meeting would not have taken place. The symposium has also been strongly supported; both scientifically and financially, by the EU Commission financed Yeast Systems Biology Network Project (YSBN, LSHG-CT-2005-018942). Our special thanks for administrative and secretarial help go to Ms. Anita Tienhaara (University of Helsinki) and Ms. Annemari Kuokka (VTT).

My warmest welcome to ISSY25!

Merja Penttilä

Research Professor, VTT

Chair of the Organising Committee

Local organising committee

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Takashi Ito, Japan
Jens Nielsen, Denmark
Stephen Oliver, UK
Hans Westerhoff, The Netherlands/UK

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Keynote lecture

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ABSTRACTS OF
INVITED PLENARY LECTURES
T1 – T17

Theoretical and practical systems biology

P. Varpela and R. Renkonen

Medicel Ltd and University of Helsinki, Finland

Systems Biology is biology, modelling and data integration. Unfortunately, these parts remain often separate or poorly integrated. Systems Biology lacks its own theoretical framework derived from general systems theory. Such theoretical framework and its practical implementation need to be generic enough to support different approaches and survive through years of new evolving methods and technologies. Efforts for the implementation of such framework will be rewarded with more efficient accumulation of new biological knowledge.

The first step towards such a framework is the classification of biological data into three generic domains: 1) Component, 2) System and 3) State data. Component data means definitions of biological components from small to large and from specific to general. Useful definitions are based on objective attributes or structural patterns or anything than can be used to identify different components. Component data is static in nature as long as definitions are static. System data describes how the components are connected to each other to form systems, where functionality can be represented and communicated in simple, but objective and computerized ways. State data means quantitative measurement values, experiment conditions and simulation results having what-, where-, and when- information that connects each value to a component, a system and a time point. State data is dynamic in nature.

Biological systems need to be localised somehow in biological universe in such a way that all real world samples can be correlated to the abstract models of each system. Component, system and state data are tightly coupled together, but still each has own attributes and degrees of freedom. This conceptual separation of data domains makes possible to systematically develop methodological paths between different data domains when new data is accumulated to a domain based on existing data of other domains. Medicel Ltd has developed a practical implementation of such a framework. It can be used as an “operating system” for Systems Biology.

New 'dimensions' in genome annotation

B. O. Palsson

Department of Bioengineering, UCSD, La Jolla, USA

Traditional Genome annotation involves the enumeration of open reading frames and their functional assignment. Currently, there are on-going efforts to identify all the interactions between these components. The resulting map of interactions effectively represents a two-dimensional annotation. It takes the form of a stoichiometric matrix, if the interactions are described with chemical equations. The formulation and properties of this matrix are detailed and how it can be used as the basis for computing allowable phenotypic functions. The issues associated with the packing of the microbial genome and the function of the interaction map in three-dimensions will also be discussed. Finally, we will go over the issue of genomes changing in space and time through adaptive evolution and describe the full re-sequencing of microbial genomes to map all genetic changes that occur during adaptation.

Yeasts illustrate the mechanisms of eukaryotic genome evolution

B. Dujon and the Génolevures Consortium

Unité de Génétique moléculaire des levures, Institut Pasteur, Paris, France

With the rapidly expanding number of genomes sequenced, the features of molecular evolution can now be contemplated with levels of comprehensiveness and precision unknown before. Comparisons between distinct organisms from a same phylum help us disentangle the distorted and superimposed traces left in genomes by the successive, numerous evolutionary events. But the pictures obtained are clearer when some of the compared organisms are also amenable to experimentation. This is the case for the Hemiascomycetous yeasts. With their compact genomes, these yeasts cover a very broad evolutionary range, made of billions of successive generations, which remained unsuspected from their similar life styles and morphologies. Several yeast species are commonly used for genetic experiments while others are of biotechnological importance or of medical concern as infectious agents. Altogether, almost three dozens of Hemiascomycetous yeasts have now been sequenced, either totally or partially, the largest number from a single evolutionary phylum of Eukaryotes.

Comparisons of chromosome maps and genome redundancies reveal that yeasts have evolved through a remarkable interplay between distinct molecular mechanisms including tandem gene repeat formation, massive genome duplication, segmental duplications and extensive gene loss. Experiments with *Saccharomyces cerevisiae* reveal how the chromosomal dynamics characteristic of evolution can lead to the formation of novel genes, whereas observations from the pathogenic yeast *Candida glabrata* simultaneously reveal the loss of function and the formation of specific genes. Transposon-mediated single gene duplication, mitochondrial DNA transfer and horizontal gene transfer have also played a specific, though quantitively limited, role in evolution. With the help of yeasts, the consequences of these various mechanisms, which have their equivalents in other organisms, are now becoming more clearly understood.

With the help of developing technologies, comparative yeast genomics will rapidly enter the field of population genomics. Several dozens of *Saccharomyces* isolates are being sequenced and compared. At the same time, a greater emphasis on the genes encoding RNA molecules is needed. Preliminary data suggests how they differ from the protein-coding genes and allow interesting speculations that are amenable to experimental testing.

Integrative genomics of fungi

J. E. Galagan

The Broad Institute of MIT and Harvard, Cambridge, USA

The availability of numerous fungal genome sequences along with growing resources for functional genomics provide an unparalleled opportunity to study the genetics and cellular biology of fungi in an integrative fashion. Although such studies have been possible for *S. cerevisiae* for some time, there is a growing opportunity for other fungi as well. In my talk I will present some of the work in my group focused deciphering the functions and evolution of genomes, genes, and gene regulatory networks in fungi.

In particular, I will present ongoing work in three areas. (1) I will describe a new framework we have developed for comparative gene prediction using Conditional Random Fields (CRFs). CRFs provide substantial advantages over current HMM based gene predictors and we have developed a gene caller using CRFs that shows comparable accuracy to the best multigenome gene predictors for fungi. (2) I will describe our ongoing analysis of genome evolution in fungi and will provide an update of our analysis of related *Aspergilli*. (3) I will present two lines of evidence, derived from our analysis of *Cryptococcus neoformans*, that in at least some cases, natural selection actively works to reduce translational efficiency.

Genome-wide identification and analysis of biological systems in yeast

S. G. Oliver

Faculty of Sciences, The University of Manchester, UK

While there are plans to construct complete mathematical models of unicellular organisms, such grand syntheses are still a long way off – not least because much of the quantitative data that will be required, if such models are to have predictive value and explanatory power, simply do not exist. Therefore, we will have to first construct models of smaller sub-systems (e.g. glycolysis, nucleotide biosynthesis, the cell cycle etc.) and then integrate these component modules into a single construct, representing the entire cell. The problem, then, is to ensure that the modules can be joined up in a seamless manner to make a complete working model of a living cell that makes experimentally testable predictions and can be used to explain empirical data. There is currently much debate about the relative merits of 'bottom-up' and 'top-down' approaches in Systems Biology; in fact, both will be needed.

It is difficult to construct an overarching framework for a model of the yeast cell when one has no idea what the final model will look like. It would be very useful to build a very coarse-grained model based on our current knowledge. However, this is a dangerous step since our current knowledge is very incomplete, with much relevant data being unavailable at present. Such a construct would very likely lead to us being in a 'can't get there from here' situation a few years down the road, where we would find it impossible to integrate specific models of individual sub-systems, which had been generated by bottom-up approaches. Thus, a coarse-grained model is certainly desirable, but it might be sensible to get the yeast cell to construct it for us, rather than make an imperfect attempt to construct it ourselves. We have chosen to use the formalism of Metabolic Control Analysis for this purpose. Therefore, we must persuade yeast to identify those components of the eukaryotic cell system that exert the greatest degree of control over the pathways in which they participate, or which they regulate. In other words, we need to identify those components of a yeast cell that exert the greatest degree of control over its rate of growth and division. In the parlance of Metabolic Control Analysis, these components would be said to have high Flux Control Coefficients.

For the bottom-up approach, the initial problem is one of systems identification. While a lot of time is currently spent debating the question: "What is Systems Biology?" Why (in an organism where we know so much about its biochemistry, physiology, and cell biology as *S. cerevisiae*) should it be a problem to identify the biological sub-systems

that must be fully characterised and built into a comprehensive model of the eukaryotic cell? This problem arises because we have previously studied these biological systems in isolation and in a rigorously reductionist fashion. Now, we must study them as parts of an integrated whole. The problem is that our current view of, say, a metabolic or signal transduction pathway is often two-dimensional (rather than four-dimensional) and is frequently poorly integrated, if at all, with other cellular pathways. Thus our view of the network of metabolic pathways may not be the same as the yeast's. In order to gain a "yeast's eye view", we have coupled flux balance analysis with both metabolomics and genetics. Although the initial aim of these approaches is the identification of the 'natural' metabolic systems of yeast, the principles involved should be more widely applicable to the problem of biological systems identification.

Further exploration of the budding yeast transcriptome

F. Miura, M. Onda and T. Ito

Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo, Japan

A thorough description of transcriptome is one of the foundations for understanding the cell as a molecular system. Toward this goal, we have launched three projects, namely full-length cDNA analysis, absolute quantification of mRNAs, and enforced activation of transcription factors. In the full-length cDNA analysis, we have read two cDNA libraries, one from S288C strain exponentially growing in the SD medium and the other from SK1 strain at meiotic stages. Both libraries were constructed using a vector-capping method, which allows one to precisely map transcription start sites based on a cap-dependent nucleotide addition phenomenon. Consequently, we identified 11,519 transcription start sites for 3,638 annotated genes. The analysis also revealed 50 novel introns including those affecting ORF annotations and those spliced alternatively, 576 novel transcripts derived from intergenic regions, and antisense transcripts for, at least, 665 annotated genes. Thus, the budding yeast transcriptome is much more complex than previously thought. It may provide a tractable model system to reveal functions of noncoding RNAs found in higher eukaryotes, and the full-length cDNA clones would serve as an invaluable resource for such studies.

For the absolute quantification of mRNAs, we developed a method for 'Genome-normalized Adaptor-Tagged Competitive PCR (GATC-PCR)' in which genome DNA serves as a standard to normalize cDNA signals. Current GATC-PCR system covers almost all of the classical ORFs (5,833 ORFs) to reveal the stoichiometric composition of a given transcriptome. We are improving the system so that it can discriminate between sense and antisense transcripts. To identify targets genes of a transcription factor using gain-of-function alleles, we developed a method for chimerization-mediated activation (CMA). We applied the CMA to all of the Zn_2Cys_6-type zinc finger proteins, the largest transcription factor family in the budding yeast, and obtained transcriptome data on 51 CMA strains. The CMA approach recalled known target genes more efficiently than the systematic ChIP-Chip analysis reported previously. It also uncovered an essential role for an uncharacterized member of this family in propionate metabolism, demonstrating its power in the analysis of novel transcription factors. These approaches would complement current yeast transcriptomics and contribute to further exploration of the transcriptome.

Understanding the quantitative physiology of a eukaryotic cell fate decision system

R. Brent

The Molecular Sciences Institute, Berkeley, California, USA

I will present work on the "Alpha Project." The overall goal of the work is to gain the ability to predict the quantitative behavior of a well-studied biological cell fate regulatory system at the level of individual cells. We are studying the well-characterized G-protein receptor coupled signal transduction pathway that governs the response of haploid MATa *S. cerevisiae* to the mating pheromone, α factor. This pathway is a prototype for regulatory networks that govern cell fate decisions in response external stimuli in higher eukaryotes. It is also sufficiently tractable to have allowed us to develop (and exploit) of numerous functional genomic "wet" experimental and computational methods; and we believe it is sufficiently paradigmatic so that successful experimental and computational tactics, and scientific findings, can be ported rapidly to other systems in other organisms.

The work is necessarily crosses disciplinary boundaries, and, in its cross-cultural complexities, may represent a laboratory or test site for methods to prosecute some kinds of future biological research efforts. I will describe some developments from recent work, including improved measurement methods. I will also describe some recent scientific findings. A good deal of recent scientific work is based on a first order characterization of the baseline quantitative physiology of this signal transduction/ decisionmaking system (Colman-Lerner et al., 2005).

Among recent findings is the beginning characterization of genes whose products may be general regulators of quantitative and dynamic behavior for many intracellular processes. During the second phase of the project, 2007–2012, we hope to extend studies of these genes to their mammalian and human homologs, and thus gain clues as to the significance of this sort of regulation of quantitative system behavior to the function of multicellular organisms.

Colman-Lerner, A., Gordon, A., Serra, E., Chin, T. Resnekov, O. Endy, D., Pesce, C. G. and Brent, R. Regulated cell-to-cell variation in a cell-fate decision system. Nature, 437, 699–706 (2005).

Dynamic modeling of cAMP signal transduction in *Saccharomyces cerevisiae*

M. Reuss[1] and D. Müller[2]

[1] Institute of Biochemical Engineering, University of Stuttgart, Germany
[2] Institute of Computational Science, Zürich, Switzerland

The pursuit of a systems-level understanding of biological processes constitutes one of the central goals of systems biology. In this work, a mathematical model has been developed, which aims at capturing not only the dynamics of cAMP-PKA signal transduction, but also its impact on central metabolismus and the cell cycle machinery. The integrated model features a modular structure where each of the processes constitutes a module of its own. The model for the cAMP signal transduction encompasses the dynamics of adenylate cyclase activation via Ras proteins and via GPRC system consisting of Gpr1 and Gpa2 in conjunction with hexokinase activitiy. Stimulation of Ras by the GEFs Cdc25 and Sdc25 as well as inhibition of Ras by the GAP activitiy of Ira proteins is considered. The model captures the activation of PKA by cAMP and the degradation of cAMP by the phosphodiesterases Pde1 and Pde2. In addition, two routes of negative feed back of PKA on its own activation are accounted for: (i) the stimulation of Pde1 activity by phophorylation and (ii) partial relocalization of Cdc25 (and adenylatcyclase) to the cytoplasm following phophorylation by PKA. While a large number of parameters was directly taken from the literature, several values had to be determined by fitting the model to data of published experiments. A comparison of the simulated response of glucose-derepssed cells to the addition of 100 mM glucose for wild-type cells and several mutant strains (Rolland et al., 2000) demonstrates a good quantitative agreement of the trajectory shape for all strains.

The second part of the lecture will focus on the description of the module for the coupling between the signal transduction module and the central carbon and energy metabolism of the yeast. Here, only a subnetwork of a full model of PKA signal transduction will be employed, which describes the activation of PKA through binding of cAMP, the resulting release of the of free catalytic subunits (Tpk) as well as the effect of PKA autophophorylation. Phosphofructkinase 2 (PFK2) is considered as an example of a metabolic target protein of PKA. Its catalytic activity is stimulated upon phosphorylation by PKA. PFK2 represents a key enzyme for the regulation of the glycolytic flux because it produces fructose-2,6-bisphophate (F26BP), a potent stimulator of phosphofructokinase I and inhibitor of fructose-1,6-bisphophatase, respectively. Modeling of this subnetwork will be shown along with experimental observations of the time course of the activity of F26BP in reponse to a glucose stimulus in glucose limited chemostate culture.

Construction and analysis of kinetic models of *Saccharomyces cerevisiae*; applications to glycolysis, cell cycle and mixed populations studies

J. L. Snoep[1, 2, 3], R. Conradie[1], C. Malherbe[1], F. du Preez[1] and H. V. Westerhoff [2, 3]

[1] Dept. of Biochemistry, University of Stellenbosch, Matieland, South Africa
[2] Molecular Cell Physiology, Vrije Universiteit, Amsterdam, The Netherlands
[3] Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, The University of Manchester, School of Chemistry, UK

Systems Biology studies aim to quantitatively relate systems properties to characteristics of the underlying components. Kinetic models are important tools in such an approach and these models must be detailed enough to capture the essential behavior of these components. The Silicon Cell project (http://www.siliconcell.net) is in line with this approach, advocating the use of detailed kinetic models based on experimentally determined parameter values at the level of the (enzyme-catalysed) reaction step. We'll demonstrate the approach via the construction of a detailed kinetic model for glycolysis of *S. cerevisiae*. Although originally developed for a steady state analysis of glycolysis this model can also show limit cycle oscillations, with synchronizations between cells.

Detailed kinetic models of large systems quickly become very complicated and a higher-level description of such models is useful for the analysis of such models. Metabolic Control Analysis (MCA) gives such a higher-level description and although it was originally developed for steady state studies it has been extended to include the analysis of dynamical systems. We've made a further extension to MCA by deriving the flux and concentrations summation theorems for dynamical systems and have applied these analyses to detailed kinetic models, obtained from the literature, of the yeast cell cycle. In addition to metabolic systems, MCA can also be applied to whole cell populations, an approach that will be illustrated for a mixed population of *S. cerevisiae* and *Gluconobacter oxydans*.

Metabolic control analysis of yeast central carbon metabolism under uncertainty

V. Hatzimanikatis

Chemical Engineering, Northwestern University, Evanston, USA

Yeast metabolism has been used extensively in scientific investigations and industrial applications. Understanding the properties of the yeast metabolic network is crucial, yet unaccomplished due to its high complexity, the different culture conditions, and the uncertainty associated with kinetic parameters. We recently developed a computational and mathematical framework using Monte Carlo method in which parameter uncertainty can be addressed through risk analysis methods. This framework was applied on the compartmentalized central carbon pathways of *Saccharomyces cerevisiae* metabolism for the identification of rate limiting steps in ethanol production from glucose and xylose.

Dynamic modeling of yeast cell stress response

E. Klipp

Max Planck Institute for Molecular Genetics, Berlin, Germany

Investigation of cellular systems is more and more supported by computational methods like bioinformatics and mathematical modeling, which is an important aspect of Systems Biology (1, 2). A frequently used method is the description of reaction systems by sets of ordinary differential equations. The structure of the equations is established based on the knowledge about the network structure, i.e. about the relevant pathways and protein-protein interactions, while the parameters are determined from experimental observations, preferentially time course measurements.

Using the power of such models, we investigate stress response processes in the yeast *Saccharomyces cerevisiae*. The adaptation of the cells to environmental changes like nutrient supply, pheromone stimulation (3) or osmotic stress (4) is mediated by signaling pathways that eventually regulate the expression of many genes. The products of such genes, in turn, regulate the metabolism or the cell cycle progression in order to compensate for or adapt to the external stimuli. The predictions of the models agree well with experimental results obtained under different stress conditions or using certain mutants. Simulations reveal properties of the signaling process and enlighten the roles of different components in the adaptation process.

The presented examples show that mathematical models are helpful to formulate experimental knowledge in a testable form, to explain hitherto unsolved phenomena and to even predict the outcome of new experiments.

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2. Klipp, E., Herwig, R., Kowald, A., Wierling, C. and Lehrach, H. Systems Biology in Practice. Wiley-VCH, Weinheim, 2005.
3. Kofahl, B. and Klipp, E. Yeast 2004; 21:831–850.
4. Klipp, E., Nordlander, B., Krüger, R., Gennemark, P. and Hohmann, S. Nature Biotechnology 2005; 23: 975–982.

Exit from mitosis in budding yeast: models and experiments

B. Novák[1], E. Queralt[2] and F. Uhlmann[2]

[1] Molecular Network Dynamics Group of the Hungarian Academy of Sciences, and Budapest University of Technology and Economics, Hungary
[2] Chromosome Segregation Laboratory, Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratory, UK

After anaphase, the high mitotic cyclin-dependent kinase (Cdk) activity is down-regulated to promote exit from mitosis. Mitotic exit in budding yeast is fully dependent on release and activation of the Cdk counteracting phosphatase, Cdc14. In metaphase, Cdc14 is kept inactive in the nucleolus by its inhibitor Net1. During anaphase, Net1 gets phosphorylated which causes Cdc14 release and activation. How Net1 gets phosphorylated specifically in anaphase has remained a mystery. Here we show that $PP2A^{Cdc55}$ phosphatase keeps Net1 under-phosphorylated in metaphase. At anaphase onset the sister-chromatid separating protease separase gets activated and down-regulates $PP2A^{Cdc55}$ activity thereby facilitating Cdk-dependent Net1 phosphorylation. We also show that $PP2A^{Cdc55}$ down-regulation contributes to the activation of the 'mitotic exit network' that sustains Cdc14 as Cdk activity declines. Based on these experiments we present a comprehensive quantitative model for mitotic exit in budding yeast. The heart of the model is the antagonistic interaction between Cdk and cyclin degradation machinery, which creates bistability and hysteresis.

Reference:

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Adaptation of *Saccharomyces cerevisiae* to a changing environment: a vertical genomics approach

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In *Saccharomyces cerevisiae* metabolism and physiology, growth rate and cell cycle progression are tightly coordinated in response to the nutritional environment (1). We have used the long-known specific adaptation of this yeast from respiring growth conditions to anaerobic, sugar excess fermentative growth to study in detail the regulatory mechanisms responsible for the required physiological transitions. A dynamic experimental set up has been devised based on chemostats, where the effects of switching of an aerobic, respiratory culture to anaerobiosis followed by pulsing of excess glucose can be followed over time. The resulting physiological responses are being monitored by measuring concentrations of metabolites and reserve carbohydrates, activities of various enzymes, as well as transcriptome and proteome profiles. Several clusters of genes were identified which specifically respond to either anaerobiosis, glucose-excess, or both. Interestingly, several genes were identified of which the regulation was not previously described in this context. The overall response is clearly bi-phasic, lending further support to the occurance of cell cycle arrest as a means of efficient physiological adaptation.

Further insight into the molecular mechanisms of fermentative adaptation of *S. cerevisiae* was obtained by studying the involvement of the zinc-finger protein Sfp1. Previous studies suggested a role as a regulator of cell size at START, and as a transcription regulator for genes involved in ribosome biogenesis (2, 3). Thus, *SFP1* would be a key element in the coupling of growth and cell cycle progression. We have analyzed the fermentative adaptation behaviour of an *SFP1* deletion strain in both steady state and dynamic experimental set ups. Results clearly indicate that yeast *SFP1* is directly involved in the control of all genes required for ribosome biogenesis and maturation, but not of ribosomal protein genes. Moreover, *SFP1* indeed is required for cell size control, but not for normal cell cycle progression. Consequences of these findings will be discussed in the context of fundamental and applied aspects of yeast fermentative adaptation.

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Application of system biology on brewer's yeast

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Yeast is important for systems biology not only as an excellent model species but also from an industrial point of view. A wealth of biological information such as genome-wide data and metabolic pathways available on the yeast *Saccharomyces cerevisiae* allows it to serve as systems biology application to an industrial brewing. Fermenting yeast cells produce a wide variety of secondary metabolites, including certain carbonyl compounds, sulfur-containing compounds, organic acids, fusel alcohols and esters. For example, hydrogen sulfide (H_2S) and sulfur dioxide (SO_2) are important sulfur compounds to flavor of beer. Many studies have been carried out to understand the physiology of the sulfur metabolism of *Saccharomyces cerevisiae*. The prerequisite for sulfite production by yeast is inorganic sulfate, which is imported through a specific permease. The sulfate is converted into adenylylsulfate and then to phosphoadenylylsulfate, which is subsequently turned into sulfite. The further intermediates, sulfide and homocysteine, lead to cysteine, methionine and *S*-adenosylmethionine(AdoMet). The system biology approach involving monitoring gene, protein and phenotype expression works is thought to be very helpful to identify the key process parameters that influence these flavor formation during practical brews.

However, there still remain some problems when the systems biology approach is applied on the brewer's yeast, especially when analyzing brewer's yeast genome or genome-wide data. The genome of brewer's yeast *Saccharomyces pastorianus*, which is thought to be hybrid strains, is more complicated than that of *S. cerevisiae*. Several studies have suggested that *S. pastorianus* contains parts of two diverged genomes from *S. cerevisiae* and *Saccharomyces bayanus*. Therefore, it is important to study the possible differential expression of the *S. cerevisiae* and *S. bayanus* alleles during fermentation in order to elucidate which genomes contribute to important flavor formation in beer. In this presentation, we will discuss the following topics on the bases of our recent results; (i) the genome of brewer's yeast, (ii) the possible differential expression of the *S. cerevisiae* and *S. bayanus* alleles, (iii) the contribution of different genomes to flavor formation.

Chemical combinations as multi-target therapeutics and biological probes

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Combination drugs can overcome compensatory mechanisms or evolved resistance by attacking disease on several fronts, and also provide a new window on biological systems. We present simulations and experiments that show the relationship between chemical synergies and target connectivity, as well as preliminary results from combination screens of targeted agents in yeast and tumor cell lines.

Despite the success of discovery efforts focused on specific targets, many drugs are less effective than expected [Sams-Dodd 2005], due to mechanistic complexity [Hartwell 2001] or evolved resistance. Network simulations [Csermely 2005] and clinical oncology [Kaelin 2005] suggest that disease is more controllable through multi-target approaches, and indeed combinations are the norm for cancer and many infectious diseases. CombinatoRx is systematically screening combinations of approved drugs in cellular disease models [Borisy 2003], to find synergies that can be optimized towards novel combination drugs.

We show that chemical combinations can also yield connectivity information through their response surface shapes. Pathway simulations with pairs of inhibitors produce distinct responses depending on how the targets are connected. The predicted shapes are reproduced in a yeast experiment, with further support from screens using human cells. While analogous to genetic interactions [Tong 2003], chemical synergies provide complementary and more detailed information for network models about connections between their protein targets. Chemicals can also be efficiently screened in disease models that are not amenable to genetic studies. We also present preliminary results from screens testing combinations of ~100 chemical probes with known targets using yeast and tumor cell lines. The experiments are designed to improve our understanding of yeast networks, and to identify new multi-target mechanisms with therapeutic potential for cancer.

Reporter features: A tool for mapping of global control in metabolism through model driven analysis of ome data

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The central metabolic network is highly conserved between species, but different regulatory structures have evolved in different species. Despite the variation in regulatory structures it is, however, important to ensure proper function of the overall metabolic network, i.e. control structures needs to be in place for ensuring that there is a tight balancing of the production and consumption of co-factors like ATP, NADH and NADPH. Through the use of a detailed metabolic network for the yeast *Saccharomyces cerevisiae* we have earlier shown that there is a global transcriptional response to genetic and environmental perturbations. Furthermore, we introduced the concept of reporter metabolites, which are metabolites in the metabolic network around which there are major transcriptional changes upon a given perturbation. We have now taken this concept further through the introduction of other reporter features, e.g. reporter GO annotations, reporter protein kinases, reporter proteins in protein-protein interaction networks, and reporter effectors. We have also extended the concept to identify reporter reactions based on metabolome data. In order to gain further insight into regulatory structures we have extended this concept further by including information about directional fluxes into metabolite pools. In the presentation these concepts will be described and the use of the concepts will be demonstrated for various sets of data.

Biological robustness

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Robustness is a ubiquitously observed property of biological systems. It is considered to be a fundamental feature of complex evolvable systems. It is attained by several underlying principles that are universal to both biological organisms and sophisticated engineering systems. Robustness facilitates evolvability and robust traits are often selected by evolution. Such a mutually beneficial process is made possible by specific architectural features observed in robust systems. But there are trade-offs between robustness, fragility, performance and resource demands, which explain system behaviour, including the patterns of failure. Insights into inherent properties of robust systems will provide us with a better understanding of complex diseases and a guiding principle for therapy design.

ABSTRACTS OF
AFTERNOON SESSION TALKS
T18 – T34

Evolutionary origin of novel short transcripts in *S. cerevisiae*

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Ten years ago, the genome sequencing project of *S. cerevisiae* led to the first gene annotation of a eukaryotic genome (Goffeau et al. 1996, Science). This information initiated an experimental revolution in whole genome approaches. The biological data obtained can only be fully exploited if the information content of the genome sequence is known as completely as possible. The sequencing of additional Saccharomyces-related genomes in the past years led to over hundred corrections in the annotations of *S. cerevisiae* genes but, in the absence of experimental data, not to a comprehensive map of transcripts (Clifton et al. 2003, Science; Kellis et al. 2003, Nature; Brachat et al. 2003, Genome Biology; Dietrich et al. 2004, Science; Kellis et al. and Dujon et al. 2004, Nature). Recently, a new high-resolution transcription map of *S. cerevisiae* was published showing over 1000 so far not annotated mainly short transcripts (David et al. 2006, PNAS). We wanted to know whether these novel transcripts can, at least in part, be explained by remnants of promoter sequences at sites of genome rearrangements which occurred in the *S. cerevisiae* lineage since its genome duplication 100 million years ago. A reconstruction of the ancestral genome prior to this duplication allowed the mapping of 300 chromosomal break points (translocations, inversions) and 4000 gene deletions which shaped today's *S. cerevisiae* genome (Dietrich et al. 2004, Science). Interestingly, the majority of these DNA break points map in inter-ORF regions raising questions about the fate of residual promoters. We will present results of a bioinformatic analysis on the overlaps of newly detected transcribed sequences and potential remnants of promoter regions which participated in genome rearrangements.

Conditional perturbations for GDP-mannose related genes in *S. cerevisiae*

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We describe here an approach to analyse the system level regulation of the fate of glucose, burned either to energy or used for cell wall synthesis in S. cerevisiae. This required first the development of wet lab tools to allow the high-throughput approaches such as sampling from bioreactor cultivations, a collection of *in silico* workflows for the data analysis as well as their integration into a large data warehouse. With the use of the above mentioned approaches we could show that conditional knocking down of genes related to GDP-mannose synthesis or transportation lead to altered gene expreesion of over 300 genes. These genes (and their corresponding proteins) were further analysed and characterized by various ways, such as GO enrichments, novel pathways were built between such GO caterories, their putative transcriptional regulation was analysed and novel protein-proten interaction networks were built between these proteins. Taken together we provide evidence that the effective use of a combination of wet lab generated in new systems biology experimentations. When these results are always added to an ever-expanding data warehouse as annotations they will incrementally increase the knowledge of biological systems.

Large-scale analysis of yeast and human integral membrane protein interactions using the membrane yeast two-hybrid approach

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Approximately one third of all predicted proteins in any living cell are localized in the membranes. Membrane proteins are involved in a wide range of essential cellular functions and, importantly, most pharmaceutical drugs developed today interfere with membrane proteins. For this reason, there is a strong demand from both academic researchers and biotech-pharma companies to gain further insight into pathways and interactions involving membrane proteins. However, the hydrophobic nature of membrane proteins often results in insoluble proteins which makes protein isolation difficult and therefore hinders the determination of protein complex composition and protein function. Previously, we have developed a yeast-based proteomic technology for the in vivo detection of membrane protein interactions, so-called membrane-based yeast two-hybrid (MbYTH) system. Our current effort is directed to the generation of a comprehensive protein interaction network of the selected yeast membrane proteins whose human homologs are involved in the onset of human diseases as well as the majority of the human G-protein coupled receptors (GPCRs). During my talk, I will report on the latest achievements on this project and will highlight the latest interaction and functional data.

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Vertical genomics: fast dynamic response of central metabolism and glycolytic genes to glucose availability in *S. cerevisiae*

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Our project aims at elucidating the interactions between the different levels of regulation of glycolysis in the yeast *S. cerevisiae*, namely allosteric interaction, covalent modification and transcriptional regulation. It is generally regarded that the different regulatory mechanisms have different characteristic times. By studying the response of yeast to perturbations over short time scales, we will be able to establish the time sequence of regulation events and clarify the relations between them. For that purpose, pulse response experiments were carried out using *S. cerevisiae* cells grown under well-defined conditions in aerobic glucose-limited chemostats. The response of the cells to a small glucose pulse was followed in terms of the intracellular concentrations of central metabolism intermediates, nucleotides and aminoacids, extracellular concentrations of metabolites and transcript levels of glycolytic and regulatory genes.

Upon a glucose pulse, the changes in fluxes in and out of the cell are instantaneous. Within few seconds, this leads to very significant changes in the intermediates of central carbon and energy metabolism. However, it was observed that the dynamics of these metabolites occurred mainly in the first 2–3 min following the perturbation, after which most concentrations stabilized to a pseudo-steady-state level. In contrast to the highly dynamic behavior of the primary metabolites, the changes in free aminoacid concentrations were much slower and less pronounced. Transcript levels were followed with a sampling interval of 1–2 min for a period of 30 min after the glucose perturbation. This allowed us to capture with considerable detail the fast dynamics of the transcripts of glycolytic genes, various regulators and other related genes. The first changes in transcript levels are observable already at 1–2 minutes after the glucose addition. The changes include several aspects of glucose repression, such as the reconfiguration of the isoenzyme pool of hexose transporters and the decrease in levels of known glucose-repressed genes. In addition, we have found responses in the genes of the hexose kinases, most of lower glycolysis, ethanol and glycerol synthesis and several genes with sensing and regulatory functions. Finally, all the transcripts with decreasing levels have half-lives significantly lower than the data available in literature, suggesting there is fast active degradation of mRNA. Experiments are under way to better describe the role of degradation in the regulation of mRNA levels under these conditions. Better insight in the concerted fast dynamics of metabolites, fluxes and transcript levels will help to elucidate the mechanisms of enzyme regulation, intracellular signal transduction and control of mRNA synthesis and degradation.

Determination of the transcriptional networks controlling pseudohyphal growth in *Saccharomyces cerevisiae* and their comparison to related yeast species

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To understand the organization of the transcriptional networks that govern cell differentiation, we investigated the binding network formed by several regulators (Tec1, Ste12, Sok2, Phd1, Mga1 and Flo8) of pseudohyphal growth in *Saccharomyces cerevisiae* using chromatin immunoprecipitation microarrays (chIP chip). The factors and their targets form a complex binding network, containing patterns characteristic of autoregulation, feed-back and feed-forward loops and cross-talk. Analysis of the network also identified Mga1 and Phd1 as key target hubs, as the promoter of each gene was shown to be bound by all of the factors investigated. These target hubs were subsequently shown to represent master regulators of this complex developmental process and expression of either Mga1 or Phd1 was capable of activating the pseudohyphal response under inappropriate conditions.

In order to further understand of the *S. cerevisiae* network and to investigate the evolution of this intricate regulatory system, the networks formed by the pseudohyphal regulators Tec1 and Ste12 were also determined by chIP chip in the related yeast species *S. mikatae* and *S. bayanus*. Comparisons of the binding networks showed that while the majority of targets are conserved between the three species, there are many examples of species-specific differences in the binding profiles of these factors. The analysis of these differences should provide insights into the evolution of the pseudohyphal growth response which differs substantially in these three species despite their close evolutionary relationships.

The time-dependent response of *S. cerevisiae* to oxidative stress

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Oxidative stress is a harmful condition in a cell, tissue, or organ, caused by an imbalance between reactive oxygen species (ROS) or other oxidants and the capacity of antioxidants and repair systems. These oxidants interact with certain biomolecules and start a chain reaction of damage among important cellular components, such as membranes and DNA. Oxidative stress is an important pathophysiologic component of a number of diseases, such as Alzheimer's disease, diabetes and cancers. Despite much accumulated knowledge about these responses, their kinetics is still not clearly understood.

Here we study the kinetics of genome-wide response of the model organism *Saccharomyces cerevisiae* to oxidative stress induced by the cumene hydroperoxide (CHP). Gene expression profiles after exposure to CHP were obtained in controlled conditions using Affymetrix Yeast Genome S98 arrays. The oxidative stress response was measured at 8 time points within 120 minutes after the addition of CHP. Because we had no information on how fast the responses would occur, we designed a logarithmic spacing for the sample collections, with the earliest being 3 minute after exposure. Results show that some genes respond very quickly with high levels of induction detected at this early time. A variety of microarray data analysis methods, including three-way ANOVA, *k*-means clustering, template matching and pathway analysis were used to analyze the data. The results from this study provide a dynamic resolution of the oxidative stress responses in *S cerevisiae*, and contribute to a much richer understanding of the anti-oxidative defense system.

Physiological response of *Saccharomyces cerevisiae* to change in oxygen provision

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Oxygen is a major determinant of cellular physiology and is of particular importance in industrial biotech processes, in which the cost of oxygen supply must be balanced with performance requirements. Bakers' yeast, *Saccharomyces cerevisiae*, is one of the most exploited industrial organisms and regardless of whether the process is aerated, as in biomass and protein manufacture, or non-aerated, as in brewing, the role of oxygen is crucial.

Saccharomyces cerevisiae was grown in glucose-limited chemostat culture with 0% (anaerobic), 0.5%, 1%, 2.8% or 21% oxygen (D = 0.10 h^{-1}, pH 5). After steady states had been achieved, the conditions were shifted from aerobic to anaerobic or from anaerobic to aerobic. During and following the shift in conditions, samples were removed and analysed for changes at the metabolite and transcript (primarily those involved in carbon metabolism) levels. Metabolites of upper glycolysis generally increased, following a transition to anaerobic conditions, while the metabolites of lower glycolysis generally decreased. Metabolites from the TCA cycle generally increased under the same conditions. Similar, opposite responses were observed when conditions were shifted from anaerobic to aerobic. Gene regulation, however, was more complex, with several genes showing transient up- or down-regulation following a change in conditions. Responses to a shift from aerobic to anaerobic conditions were generally similar, regardless of the oxygen concentration prior to the shift. Based on these results, biomarkers will be identified which can be used in assessing oxygen provision to *S. cerevisiae* cultures.

Monitoring fast dynamic combined response metabolome and transcriptome of *S. cerevisiae* to a glucose pulse

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Yeast cells are known to respond to environmental perturbations via multiple levels of regulation. For example, a sudden excess of glucose triggers fast changes in the intracellular metabolite levels of *Saccharomyces cerevisiae* that are shortly followed by a major transcriptional reprogramming that, in its turn, induces changes in the enzyme levels. In this study the fast transient response (0–360 s) of *S. cerevisiae*, cultivated in a glucose limited chemostat culture, to the glucose pulse perturbation was studied with respect to metabolome and transcriptome dynamics.

The results, which were verified in multiple independent experiments, include a highly resolved timing of the initiation of the transcriptome responses. In addition, the approach enables the quantification of the half-life of the down-regulated transcripts, which reveals a striking 10-fold decrease in the average half life compared to a previous study (Wang *et al.*, 2002). This observation supports the hypothesis that transcript levels are regulated both by the transcription and the degradation process.

Furthermore, the integration of metabolome and transcriptome data discloses tight relationships between the metabolic and transcriptional changes. As an example, a significant drop in the adenine nucleotide pool following a glucose pulse was accompanied by a concerted upregulation of genes in the purine biosynthetic and salvage pathways. In our view the combined analysis of multi-omics dynamic data obtained in well-controlled perturbations is indispensable for the detailed study of the cell physiology. It yields the experimental input that is mandatory for improving and validating models in a systems biology approach (Kitano, 2002).

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Automated flow cytometry for studying population dynamics

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An important goal in systems biology is to establish the relationship between the genetic information of an organism and the cell function at different levels of biological organization. Cell function at the population level represents the most complex level of organization of single-cell organisms as it is reflected in cellular property distributions that are influenced also by the cell environment. The study of the cellular phenotype at this level requires the quantitative evaluation of properties of individual cells in the context of the surrounding medium. We have developed automated flow cytometry as a new tool to investigate dynamic properties of cell populations in great detail. A useful application of this approach is the cytostat, a controlled continuous cultivation technique which permits accurate determination of specific growth rates and property distributions of cell populations in a precisely defined environment. In a cytostat, an automated flow cytometer system monitors the single cell phenotype and can control the continuous culture even at very low cell concentrations. These few cells, present in the reactor, negligibly change the bioreactor medium composition. Therefore, the cell environment is precisely defined by the feed composition. The cytostat provides a convenient way to determine the effects of the growth medium on the dynamic properties of a growing culture and permits identification of parameters of the population balance equation which offers a rigorous theoretical framework to describe the dynamics. Moreover, operation of the cytostat at very dilute cell density eliminates effects caused by products of cell growth. For instance, we have shown that the increase in cell size of *Saccharomyces cerevisiae*, usually attributed to faster specific growth rates, is caused by the increased ethanol concentration typically encountered in high density growth experiments. By appropriately implementing the cytostat at low cell densities, important physiological problems can be probed under well-controlled conditions. Cells grown under steady state conditions within a cytostat can then be analyzed using traditional systems biology techniques including microarrays in order to relate the precisely defined growth conditions to the global expression characteristics of cells.

Deciphering condition dependent metabolic regulation in *Saccharomyces cerevisiae* by ^{13}C flux analysis

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Large-scale flux analyses highlighted that the distribution of metabolic fluxes in a given organism under a given environmental condition is rather robust to random genetic perturbations (Blank et al., 2005; Fischer & Sauer, 2005). And increasing evidence suggests that the characteristic organism/condition-specific distribution of flux is controlled through the underlying regulatory network. Here, we investigated by metabolic flux profiling the global response of a genetic perturbation in the major metabolic regulation pathways. As metabolic flux analysis is concerned with the quantification of carbon fluxes in central carbon metabolism, we chose regulators which are involved in phosphate- , nitrogen- , and glucose metabolism plus major regulators of osmotic- and aerobic stress, and of unknown function. Results on the measured metabolic responses of the regulatory mutants in dependency of the growth environment will be presented and will be discussed in the context of the regulatory network of S. cerevisiae.

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Functional implications of changes in gene expression

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Functional behavior of cells largely occurs at the level of 'fluxes', i.e. rates of processes such as product formation, protein production/degradation and gene expression. Although the mechanisms of these processes are known in great detail, it has not yet been possible to predict how changes of transcription eventually affect metabolic fluxes. We hypothesize that this is due to simultaneous regulation of all processes involved. Thanks to genomics, it should be possible to evaluate the implications of processes at the level of transcription for functional fluxes. Glycolysis in yeast is a good model system to test this relation, as it is one of the few pathways for which the kinetic properties of the enzymes are known sufficiently to calculate the flux from the enzyme activities and yeast can be brought under the well-defined steady-state and transient conditions.

In the project steady-state yeast cultures are perturbed in different ways (for instance by shifting from aerobic to anaerobic conditions, by temperature-shift and by shifting from respiratory to fermentative growth). Using regulation analysis (Rossell et al., 2004) we will quantify to what extent these changes are caused by changes in transcription, translation and/or metabolism. Concentrations of RNA, protein and metabolites, and enzyme activities and fluxes are measured quantitatively. Cultivation as well as sample analysis is done in 6 different labs. Therefore, standardization is a key issue in this project.

Comparative computational modelling reveals a novel logic of tor signalling in yeast

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Quantitative computational interpretation of experimental observations has successfully revealed 'missing links', essential features, and design principles of complex cellular control circuits, mainly by establishing single mathematical representations for well-characterized systems. However, model development suffers from limited mechanistic knowledge, conflicting hypotheses, and scarce experimental data. This appears prohibitive for addressing many areas of biology and computational methods for this daring problem are lacking. Here, we show that a conceptually novel comparative computational biology approach, when combined with targeted experimental analysis, can unravel key operating principles in complex cellular pathways despite high uncertainty. It relies on systematically casting molecular hypotheses into families of mathematical models, rigorously relating model validity to the capability to describe the available experimental data, and identifying pivotal experiments to discriminate between alternative hypotheses. For a proof-of-concept, we constructed a library of dynamic mathematical models that represent different molecular mechanisms for the interaction of components in the TOR (target of rapamycin) pathway of budding yeast, a highly conserved pathway that couples nutrient availability to cell growth. In contrast to the prevailing view of a *de novo* assembly of type 2A phosphatase complexes (PP2As), our integrated computational and experimental analysis proposes kinetically controlled formation of a complex specificity factor for PP2As as key signalling mechanism that is quantitatively consistent with all presently available experimental data. In particular, we experimentally validated key model predictions, which revealed unexpected interactions between PP2As and the regulator Tip41p. Combined evidence from theoretical and experimental analysis also points to a critical role of Tap42p-Tip41p complex formation in signalling towards the transcription factor Gln3p. Beyond revising our picture of the TOR pathway, we expect comparative modelling to help in elucidating other insufficiently characterized cellular circuits.

Metabolic engineering of yeasts for production of bulk fermentation products from xylose: ethanol and lactic acid

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Kluyveromyces marxianus, a yeast naturally assimilating but not fermenting xylose, was genetically engineered to produce ethanol from xylose efficiently. Genetic engineering included replacing the natural xylose utilization pathway via xylose reductase and xylitol dehydrogenase to xylulose by a fungal xylose isomerase converting xylose directly to xylulose. Furthermore xylulokinase was overexpressed to improve efficiency of xylose to ethanol fermentation. The resulting strain produced 37 g/L ethanol with a yield of 0.4 g/g xylose used and an ethanol production rate of 0.94 g/L*h in shake flask fermentation tests. The original strain produced xylitol very efficiently in these conditions but did not produce any ethanol.

The above strain was further engineered to produce lactic acid from xylose by expressing the L-lactate dehydrogenase (LDH) from *Lactobacillus helveticus*. The resulting strain produced lactate from xylose in shake flask test conditions with 77% yield and 1 g/L*h productivity.

Lactobacillus helveticus LDH was also introduced to *Pichia stipitis*, a yeast naturally fermenting xylose. Lactate was the main product from xylose in shake flask test and was produced with about 60% yield and 0.4 g/L*h productivity.

L-ascorbic acid production from D-glucose in metabolic engineered *Saccharomyces cerevisiae* and its effect on strain robustness

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L-ascorbic acid is a molecule utilised in several industrial fields such as food, pharmaceutical, and cosmetic and as an additive in fodder. In 2002, the world production was estimated at approximately 80000 tons, with a market of $600 million. L-ascorbic acid has been industrially produced for around 60 years by a primarily chemical process based on D-glucose as the starting substrate (*Reichstein* synthesis). Despite the advent of rDNA technology and the broader utilization of microorganisms as cell factories allowed the implementation of the chemical synthesis with biotransformations, still the process implies a multi-step procedure and the utilization of different toxic compounds. For said reasons, it is desirable to develop a single-step process that could at the same time be less expensive and more sustainable.

Metabolic engineering, thanks to the targeted optimisation of cellular activities and functions, seems to be the more suitable approach to face this challenge. Here we present our recent data regarding the construction of a recombinant *S. cerevisiae* strain able to covert D-glucose into L-ascorbic acid, naturally not produced by yeasts. This result was obtained by implementing endogenous enzymatic activities with heterologous ones, by the (over)expression of the known plant genes of the pathway. Remarkably, the partial redirection of D-Glucose to vitamin C was observed despite one activity is still missing for the completion of the desired pathway. Even more remarkably, thanks to the improvement of the intracellular antioxidant levels, the engineered strain becomes much more tolerant to different environmental constraints, such as hydrogen peroxide, temperature. Moreover, by microscopic and flow cytometric analyses performed on cells grown under oxidative stress condition, we could correlate the higher intracellular antioxidant levels of the engineered strains with a lower accumulation of ROS (Reactive Oxygen Species) and with an higher cell viability. We strongly believe that this aspect of "cell robustness" can be crucial for the host optimization, considering that very often industrial processes imply severe chemical and physical constraints to the chosen cell factory.

Keywords: ascorbic acid, metabolic engineering, yeasts, strain robustness

Metabolic engineering of *S. cerevisiae* for overproduction of succinic acid

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Central carbon metabolism in *Saccharomyces cerevisiae* has been extensively investigated using a wide variety of substrates for determination of how glycolytic flux is distributed across C_1 ($CO_{2,g}$), C_2 (glycerol, ethanol, acetate), and C_3 (pyruvate) products. The distribution of carbon of a fermentable carbon-source is controlled via inter-connected regulatory signalling cascades, such as those governing substrate availability/uptake, gene repression/expression, and reduction-oxidation co-factor balance. For the *S. cerevisiae* CEN.PK113-7D strain cultivated under carbon-limited, aerobic, well-controlled batch fermentations, the distribution of carbon across biomass, C_1, C_2, and C_3 products is 18, 14, 62, and 0.5 C-mol/C-mol-glucose, respectively, with <5 C-mol/C-mol glucose unaccounted for. In the last decade, significant metabolic engineering efforts have been explored to enhance *S. cerevisiae's* propensity for C_2 production, primarily driven by the demand for bio-ethanol. However, with the advent of industrial biotechnology searching to expand biomass based production of high value-added chemicals and intermediates, enhanced production of higher carbon products is desired. Given the relatively extensive genomic, transcriptomic, proteomic, and metabolomic characterization of *S. cerevisiae*, both *in vivo* and *in silico*, it may serve as an ideal microbial cell factory for C_4 organic acid production. Succinic acid, presently produced from the petro-chemical conversion of maleic anhydride, is considered a critical building block for a wide variety of high added-value chemical commodities. Succinic acid is produced in prokaryotes and eukaryotes in the TCA cycle by GTP coupled oxidation of succinyl-CoA, and as a by-product of the isocitrate lyase catalyzed conversion of isocitrate to glyoxylate. There are several biomass platforms, all prokaryotic, for succinic acid production, including *Anaerbiospirillium succiniproducens*, *Actinobacillus succinogenes*, and *Mannheimia succiniciproducens*. However, overproduction of succinic acid in *S. cerevisiae* offers distinct process advantages, while also serving as a case study for elucidation of how glycolytic flux and signalling cascades may be modified for enhanced production of higher carbon products. The research presented here proposes several metabolic engineering strategies based on *in silico* predicted gene target deletions, and demonstrates in well-controlled fermentations that overproduction of succinic acid is feasible.

Comparative ^{13}C flux analysis of two *Saccharomyces cerevisiae* strains reveals substantial differences in the flux through the PP pathway

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A high level of biodiversity in metabolic pathways can be expected between strains specifically adapted to industrial conditions and other *S. cerevisiae* strains. The quantification of intracellular metabolic flux from ^{13}C glucose, which has recently emerged as a key approach for analysing the in vivo flux distribution, was used in this study for comparing the central carbon metabolism of the *S. cerevisiae* laboratory srain ENY and a wine yeast (V5), selected for its specific properties. In *S. cerevisiae*, deletion of *PGI* encoding phosphoglucose isomerase abolishes growth on glucose as sole carbon source[1]. However, growth of ENY *pgi* was restored by expression of the soluble transhydrogenase from *Escherichia coli*, which indicates that the pentose phosphate pathway (PPP) has the capacity to exclusively catabolize glucose, provided that the surplus of NADPH is reoxized[2]. Surprisingly, we recently found that the growth defect of a *S. cerevisiae* V5 *pgi* mutant (wine yeast background) was not rescued by expression of soluble transhydrogenases (*E. coli*, *A. vinelandii)*. Furthermore, the intracellular NADPH concentration was 5-fold lower in V5 compared to ENY. These data suggest a lower capacity of the PPP in the wine yeast compared to the lab strain.

The intracellular carbon fluxes of the two strains were quantified by mass isotopomer measurements and using the metabolic flux model from Gombert et al[3], adapted for anaerobic continuous cultivations that mimic the growth phase of wine fermentation (glucose excess). The metabolic network analysis showed substantial changes in flux distributions, of which a considerably lower flux through the PPP in the wine yeast compared to the lab strain. Since the PPP activity is driven by the demand for NADPH, the observed differences may be caused by a different requirement for biomass formation[4]. Whereas V5 had a higher biomass yield than ENY, significant variations were found in the biomass composition of these strains. This might explain, at least partly, differences in NADPH requirement. This work shows that metabolic-flux analysis is a powerful approach for comparative profiling of central carbon metabolism in yeast strains, thus offering new perspectives to address the metabolic biodiversity within the *S. cerevisiae* species.

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Zero growth product formation in *Saccharomyces cerevisiae*

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Biotechnological processes mainly differ from chemical processes in the fact that self-replicating catalysts, micro-organisms, are used. This self-replicating nature of micro-organisms can be a disadvantage when the desired product is not (derived from) biomass. For instance in industrial bio-ethanol fermentations biomass is the major by-product. Therefore it can be beneficial to decouple growth from product formation, to obtain a higher product yield. The mechanisms by which product formation is coupled to growth are complex and largely uncharacterised. This project aims to investigate zero growth product formation in *Saccharomyces cerevisiae.* Cultivation of the yeast will be under well controlled conditions and zero growth state will be analysed with a range of "-omics" approaches.

ABSTRACTS OF
POSTER SESSION I
Posters P1 – P55

DOME: a database and analysis system for functional genomics studies

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Advances in transcriptomics, proteomics, and metabolomics have led to a wealth of data that may aid our understanding of cellular and biochemical systems if it is appropriately integrated. We have developed a database and analysis system, DOME, which integrates data obtained at the three main levels of cellular variables: transcripts, proteins, and metabolites. DOME is a project- or laboratory-level database, rather than a large data repository.

DOME is based on a relational schema that represents details of the experimental process and of the biological organization of the target system. It follows a client-server architecture, with a client based on web browsers, and server based on PostgreSQL and applications written in PHP, PERL, C++ and R. There are four classes of users: *project managers* are allowed to define experiments through metadata describing the experimental flow; *project members* can upload data, and *readers* can only query data. Access to data query by *anonymous users* can be provided. Data queries are constructed through a simple interface that gradually guides the user to create complex SQL, though she never sees the actual SQL code, thus requiring minimal or no training. Query results can be exported in tabular format or they can be visualized directly within DOME. Currently the following visualization and analysis methods are available: color-aided table format, visualization on network diagrams (using our software BROME, described previously), principal component analysis, and *k*-means clustering. Data can be output directly or as summaries of biological replicates in the form of means and/or their standard deviations (per sample), mean-centered values (across samples), and normalized (Z-score). DOME has been used to manage data from projects we have been involved in, using *Medicago truncatula*, *Vitis vinifera*, and *Saccharomyces cerevisiae.* The software, however, operates independently of the species being analyzed.

Methods to Compare Fungal Genomes

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In what way do fungal genomes differ from those of other eukaryotes or prokaryotes? What are the "genomic signatures" that can be used to distinguish fungi? Currently there are more than 30 different fungal genomes that have been sequenced and are publicly available, and the number will continue to grow as sequencing technology becomes better and less expensive. There are many methods for comparison of these genomes, including phylogenetic trees (based on rRNA or genes), Blast matrices of proteomes, codon and amino acid usage, and repeats. Also the genomes can be compared in terms of protein families as well as the predicted localisation of proteins, such as secreted proteins, or proteins predicted to be in the Golgi, mitochondria, nucleus, etc. By combining these methods it is possible to build up an overview of the current genomic information.

Fungalweb: exploring knowledge-based bioinformatics

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The FungalWeb project is exploring the application of ontologies, the semantic web, and intelligent agents to the field of fungal genomics, enzymology, and industrial applications of enzymes. We have developed (i) a data warehouse genomes, genes, proteins and enzymes from fungi with their features and classification terms in EC, GO, InterPro, and KEGG; with mappings between the classification schemes; and homology information for proteins; (ii) a formal ontology in OWL which integrates the above concepts, has additional concepts on small molecules and taxonomy, and includes commercial vendors, products, and applications; (iii) a suite of tools in Java for probabilistic relational models with an application to inferring gene regulation from microarray data, binding sites, and functional annotation; (iv) tools for extracting relevant paragraphs of the scientific literature as applied to enzymology; and (v) several scenarios of application for FungalWeb.

E-fungi: an e-science infrastructure for comparative functional genomics in fungal species

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Classical comparative genomics identifies similar sequences in different organisms and infers functional equivalence from that sequence similarity. Comparative functional genomics aims to extend such comparisons by including experimental data about function and phenotype. In order to facilitate these comparisons, it is essential that sequence and functional data are organised in a way that makes integrated analyses straightforward. At present this is not the case; biological data is stored in a variety of formats at different sites, making integration problematic. We have previously developed the Genome Information Management System (GIMS), an integrated data warehouse storing sequence and functional data for the budding yeast *Saccharomyces cerevisiae*. The e-Fungi project will extend the functionality of GIMS to contain multiple fungal genomes, allowing cross-species analyses. The e-Fungi data warehouse currently contains genomic sequence data from twenty three fungal species. These include model organisms, non-pathogenic fungi and both human and plant pathogens.

In order to demonstrate the effectiveness of the e-Fungi approach to data management we are conducting a detailed exploration of fungal genomes. Protein sequences have been compared using all vs. all Blastp. The sequences have then been clustered on the basis of their sequence similarity. Phylogenetic trees have been generated using universal protein clusters (which contain proteins from all the fungal genomes) and using Dollo parsimony. The clusters have been further analysed allowing us to identify among other things, differences in the genomes of *Saccharomyces sensu stricto* species, the distribution of orthologs of essential and non-essential *S. cerevisiae* genes, and examples of horizontal gene transfer.

We intend to make our database queries available for use via a web interface. The first public release of the e-Fungi database in summer 2006 will provide canned queries for sequence analysis. A second release scheduled for November 2006 will provide queries relating to functional data.

Comparison of protein coding gene content of yeast and other fungal genomes

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Despite the extensive research the exact function many yeast genes remains unknown. Comparisons to other fungal genomes can add power to the genomic analysis by providing the evolutionary context of genes. Our goal is to compare the protein coding gene contents of fungal genomes and relate the differences to the physiological differences between species and taxonomic groups.

We have produced consistent Interpro annotations and clustering of protein coding sequences of 16 sequenced fungal genomes of which 8 are yeasts. Our computational system is based on BioPerl scripts and BioSQL schema for storing the sequences and annotations in a relational database. The clustering of protein sequences is done with Tribe-MCL graph clustering software using distances based on Blast E-values.

We have discovered that the number of genes belonging to protein clusters having members from all fungal species studied is negatively correlated with genome size, i.e. larger fungal genomes are likely to have more specialized functions not present in species with smaller genomes. In contrast, based on protein clustering Pezizomycotina and Saccharomycotina seem to differ in their level of paralogy, i.e. in number of duplicated genes. In Saccharomycotina average level of paralogy is positively correlated to the size of the genome. In Pezizomycotina, possibly due to Repeat Induced Point mutations (RIP), no clear correlation exists.

Using Generic Genome Browser we have created a web-based system that allows the scientists at VTT to easily utilize comparative information in their work. In particular, we link the Interpro entries and clusters so that a user can for a particular protein family browse the neighborhood of the family members detected by Interpro to assess the true extend of the family. In addition, a user can easily find the clusters and Interpro entries that have interesting species distributions. We also link the *S. cerevisiae* metabolic model iND750 to the comparative data.

Rapid gene expression analysis for evaluation of physiological state of micro-organisms

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Micro-organisms used in various types of biotechnical processes encounter constantly changing environmental conditions, to which they adapt by changing their cellular physiology. The performance of the used micro-organism has a major impact on the performance of the biotechnical process, and as a consequence, bioprocess monitoring and control strategies based on the physiological status of the cells culture have become more popular. Transcriptional analysis of selected marker genes is a potential way for robust monitoring of physiological events. However, tools suitable for high-throughput expression monitoring of process-relevant genes are scarce. We have applied a novel transcriptional analysis method called TRAC (TRanscript analysis with aid of Affinity Capture) for frequent expression analysis of a focused gene set in a wide range of fungal fermentations. TRAC method allows fast expression analysis of dozens of mRNAs from crude cell lysates by solution hybridisation with a pool of target-specific oligonucleotide probes of distinct sizes that are identified and quantified by capillary electrophoresis. The assay procedure has been semi-automated for simultaneous treatment of 96 samples using a magnetic bead particle processor. The whole assay procedure can be performed in few hours, implying its usefulness in bioprocess monitoring and control.

We have used TRAC for instance to monitor the physiological state of the filamentous fungus *Trichoderma* during protein production processes and *S. cerevisiae* physiology in various chemostat cultures and brewing processes. By process relevant gene markers it is possible to evaluate the growth and extracellular protein production potential of the culture as well as physiological responses to various stress conditions such as lack of nutrients and oxygen or limitation of the protein folding capacity. TRAC also provides a quick and affordable means to monitor transient situations, and to address the quality and stability of e.g. chemostat cultures prior to selecting samples for more thorough and expensive systems-wide analyses.

Specificity of topology of protein-protein interactions of the yeast

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The yeast protein-protein interaction network (PPI) is one of the best-annotated biological networks. To reveal the hierarchical structure of it, we investigate the network with respect to the interactivity of proteins. The protein-protein interaction data of Saccharomyces Cerevisiae were acquired from the Comprehensive Yeast Genome Database at the Munich Information centre for Protein Sequences (MIPS), 4610 proteins and 8972 interactions). In order to exclude false positive interactions, we assign localization of proteins by the experimentally confirmed localization data. Then, we reassigned interactions restricting if both proteins belong to the same area and obtain the network of 1850 proteins and 2520 interactions. For the annotating of proteins, the Saccharomyces Genome Database and function prediction in the MIPS are referred, and for cellular localization of proteins, the experimental data by Huh is used. We ignore the direction of bait-prey interactions.

We develop the method to stratify the network with respect to the number of interactions by generating networks whose interactions are $n \pm D$ only, where D is empirically fixed to 6 and n is changed from 7 to 50. By using this method, we discover that the networks, whose interactions are 7 to 36 (n = 1…30), are densely interconnected with each other. We will call the network as the middle layer, while less than 7 as the low layer and more than 43 as the high layer. Since the proteins in the middle layer are likely to interconnect with each other, the proteins in the high layer were forced to be connected to the low layer. Therefore, the ratio of connections between the high layer and the low layer is enhanced, while between the middle layer and the other layers are suppressed. We investigate the biological significance of the middle layer. We examine the ratio of the number of proteins that are relating to the formation of protein complexes. In cytoplasm and nucleus, the proteins in the middle layer show the highest relevance for forming protein complexes, compared to the other layers. To examine the evolutional significance of the middle layer, we compare the topology of protein-protein interactions among the yeast, worm and fly and confirm that the middle layer is conserved in every spice. Therefore, we believe that the middle layer is essential not only for the yeast's PPI but also for others.

Analysis of Gap1 alleles constitutively active for signaling

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Previous work has shown that the C-terminal tail of the general amino acid permease, encoded by *GAP1*, has a special role in the signaling process. Truncation of the last 14 or 26 amino acids resulted in the creation of the Gap1deltaC6 and Gap1deltaC9 alleles respectively. These two alleles have the unique property that they affect the typical PKA targets even in the absence of a nitrogen source. During conditions of nitrogen starvation in the presence of sufficient glucose, cells expressing these Gap1 alleles do not show a high trehalose- and glycogen content or expression of STRE-controlled genes. In addition, amino acid-induced trehalase activation in these strains is greatly reduced, probably due to feedback inhibition. Transport activity of Gap1, however, was only slightly increased in these mutant strains (150% and 130% compared to the wild-type in the Gap1deltaC6 and Gap1deltaC9 alleles respectively). Furthermore it was demonstrated that PKA is involved in mediating the effects caused by these alleles, since deletion of merely one of the catalytic subunits resulted in a wild-type phenotype.

To further elucidate a possible role for known nutrient-signaling pathways in mediating the effect of the constitutive Gap1 alleles, the effect(s) of blocking these pathways through the use of either specific inhibitors, strains deleted for a gene encoding an activating protein, as well as strains over-expressing an inhibitory protein, were determined.

Yeast PDK1 orthologues as putative activators of the nutrient-regulated kinase Sch9

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In mammalian cells, the PDK1 protein kinase controls a myriad of processes through substrate phosphorylation on a threonine in a conserved sequence motif (PDK1 site). The yeast *Saccharomyces cerevisiae* contains three PDK1 orthologues: Pkh1, Pkh2 and Pkh3. Combined deletion of Pkh1 and Pkh2 is lethal due to a cell wall defect. Pkh3 was isolated as a multi-copy suppressor of this defect in a *pkh1^{ts}pkh2delta* mutant at the restrictive temperature (Inagaki *et al.*, 1999).

Pkh substrates identified so far include Ypk1, Ypk2 and Pkc1, all of which are phosphorylated at their PDK1 site. This site is also present in Sch9, the yeast Akt/PKB orthologue, which is indispensable for nitrogen-induced activation of the trehalose degrading enzyme trehalase. Other kinases containing the PDK1 site are the catalytic subunits of PKA (encoded by *TPK1, TPK2* and *TPK3*). Whereas in mammalian cells PKB is a well-established PDK1 substrate, PDK1 phosphorylation of PKA still remains enigmatic.

Here we show that Pkh1 interacts with both Sch9 and Tpk1 *in vitro* and is capable of phosphorylating them *in vitro* on their PDK1 site. The strong reduction in nitrogen-induced trehalase activation in a *pkh1^{ts}pkh2delta pkh3delta* mutant and the effects of mutating the PDK1 site in Sch9 also point to the Pkh kinases as upstream regulators of this nutrient-controlled kinase. Our current research is focused on elucidating the signalling components upstream of Pkh/Sch9 as well as investigating the effects of mutating the PDK1 site in Tpk1.

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Characterization of the mRNA decapping enzyme Dcs1 as a specific inhibitor of trehalase

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Trehalase (Nth1) is a classic downstream target of cAMP dependent protein kinase (PKA) in the yeast *Saccharomyces cerevisiae*. Although *in vitro* phosphorylation of trehalase by PKA results in an activated form of the enzyme, there is no *in vivo* evidence for direct phosphorylation of trehalase by PKA. The mRNA decapping enzyme Dcs1 was proposed as an inhibitor of trehalase activity. Deletion of *DCS1* results in a high basal trehalase activity and very low trehalose content in nitrogen deprived cells. In contrast to wildtype nitrogen-deprived cells, a Dcs1 deletion mutant fails to activate trehalase in response to a nitrogen source. Overexpression of Dcs1 has the reverse effect: low basal trehalase activity and wildtype trehalose content. Dcs1 and Nth1, two cytosolic proteins, interact in the yeast two hybrid system. In addition the nutrient-sensitive DYRK kinase Yak1 has been shown to phosphorylate Dcs1. Our current work focusses on the elucidation of the precise mechanism how two distinct protein kinases, PKA and Yak1, control the threhalase enzyme in response to nutrient availability.

Discovery of the islet specific MTAC protein as a suppressor of a glucose-sensing deficient yeast mutant

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In the yeast *Saccharomyces cerevisiae* combined deletion of the genes encoding the glucose-sensing G-protein coupled receptor Gpr1 and the closest yeast homolog of mammalian PKB, Sch9, is synthetically lethal. We have screened a cDNA library of a mouse pancreatic beta cell line for suppressors of this growth defect and in this way isolated mammalian PKB as well as genes that activate the yeast cAMP-PKA pathway. Of three other genes with unknown function, one encodes a novel putative transcription factor, which we called MTAC, the 'Mammalian Transcription factor Activating the cAMP pathway in yeast'. Its expression in yeast enhances the cAMP level and this effect is dependent on Ras2. MTAC contains a high mobility group motif and localizes to the yeast nucleus. It acts by itself as an activating domain in the yeast two-hybrid system. Expression of the MTAC gene in mammalian tissues is highest in pancreatic islets. Tissue staining with pancreatic sections reveals specific expression in beta cells and alpha cells. Real-Time PCR experiments show that, in a glucose-sensitive mouse cell line, the expression of MTAC at the transcriptional level is influenced by the extracellular glucose concentration. These results demonstrate that yeast glucose-sensing mutants can be used to identify novel mammalian genes with a possible specific function in glucose-sensing cell types. Our current research is focused on elucidating the mechanism of MTAC in yeast as well as in pancreatic beta cells.

Genome-wide analyses of haplo-insufficiency and -proficiency to construct a coarse-grained yeast cell model

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Large-scale analyses are a fundamental approach in the effort to proceed from genome sequences to systems biology. Quantitative screening in yeast allows the assessment of phenotypic effects due to the deletion of one of the two homologous copies of a gene in diploid cells. When a diploid that is heterozygous for the deletion of a single gene shows an increase or decrease in its growth rate, it is said to exhibit a *haploproficient* or *haploinsufficient* phenotype. In the yeast deletion collection, two unique 20-base oligomer sequences serve as molecular 'bar codes', uniquely identifying each deletant strain. The combination of molecular bar-coding with hybridisation-array technology enables the assessment of the relative competitiveness of all 6,000 mutants in parallel, and to rank them according to their growth rate.

Population profiling data of 5,918 *Saccharomyces cerevisiae* deletants grown in chemostats under N-, C- and P-limited conditions has been analysed. An overrepresentation of ORFs exhibiting haploinsufficiency has been observed on chromosome III across all three nutrient limitations; the mechanisms triggering this biological phenomenon remain under question. A highly significant proportion of genes involved in the COPI vesicle coat and in retrograde transport, Golgi to endoplasmic reticulum, displaying a haploinsufficiency phenotype across all three nutrient-limited conditions. Impaired protein traffic from the Golgi back to the ER is probably due to the loss of the essential coatomer functions. A core set of genes exhibiting haploproficiency under N-limited media encode components of the ubiquitinylation pathway and the 26S proteasome. This may reflect an autoregulatory reaction to reduce the rate of protein turnover, thus promoting the survival of yeast cells exposed to N-limitation. Having identified these classes of genes with, in the terms of Metabolic Control Analysis, high flux-control coefficients, we intend to model these pathways for protein turnover and transport.

Analysis of metabolic network robustness in *S. cerevisiae*

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The common observation that knockout mutants have no detectable phenotype led to the concept of gene network robustness. We identified flexible reactions in yeast metabolism, i.e. reactions which are active but not essential using a genome-scale metabolic network model (Kuepfer et al., 2005). The algorithms used in this work include flux balance analysis and minimisation of metabolic adjustment. Specifically, for all genes considered in the metabolic model, a corresponding deletion mutant was experimentally analysed in minimal media with different carbon sources. The experimental conditions were chosen to guarantee a wide spectrum of different metabolic states that activate as many biosynthetic pathways as possible. By correlating our computational results with in vivo flux data determined using a metabolic flux ratio method, we show that robustness in flexible reactions is predominantly achieved through redundancy, i.e. the high number of duplicate genes present in yeast metabolism (Blank et al., 2005).

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Stress in yeast knockout experiments

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Knocking out genes is a common way to study their functions and gene regulatory networks. It is hoped for that disabling a gene changes expression levels in the same regulatory pathway. However, the very invasive knocking-out procedure may start other processes not directly related to the knocked-out gene. In particular, the yeast stress survival machinery may activate, and it may overwhelm the primary effects of the knockout. We investigate the potential presence of stress in yeast knockout experiments with bioinformatics methods. Based on our findings, we suggest that the stress reaction may have a notable contribution in knockout experiments on gene expression level. We argue that knowing whether the knockout has lead cells to a stressed state is critical for the interpretation of the results.

The stress reaction in cells is not yet quantitatively well-known, and it is not a trivial task to build a statistical model for it. We resort to a novel data-driven approach that builds on the relationships between several gene expression data sets. We assume that statistical dependencies between properly controlled stress data and knockout data are signs of the stress-like behaviour in knockout experiments.

Dependencies are sought between a public knockout expression data set and public stress data sets with a combination of generalized canonical correlation analysis (gCCA), traditional CCA, and traditional principal component analysis (PCA). These classical methods are applied in a novel combination and the possible overfitting of the models to noisy microarray data is taken into account.

Our primary result reveals a set of knockout experiments that seem to induce a stress reaction in yeast cells. Further analysis of pathways that are affected by knocking out the gene, before and after the removal of the stress, show how the interpretations of the expression data change. We additionally introduce methods for statistical removal of the stress response from the knockout experiments.

Dynamic network topology changes as result of cellular stress

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The topology of metabolic or protein-protein interaction networks has been an extensively studied subject. Our primary interest on the topic is how the context, such as changing physiological state of the system, affects the network topology and connectivity within- and between the cellular functional modules and through this obtain better understating about the control mechanisms of biological systems. In this study we investigated the changes in network structure as results of oxidative stress. We collected *S. cerevisiae* data on protein-protein interactions (DIP), metabolic pathways (KEGG), gene regulatory relationships (TRANSFAC) into our bioinformatics system [1]. We collected available experimental gene expression data from *S. cerevisiae* during oxidative stress response at different time points [2]. The data was integrated into the network context by defining criteria for evaluating presence or absence of proteins in the integrated network. Swissprot index of *S. cerevisiae* proteins [3] was used to translate ORF identifiers into the expression dataset to Swissprot protein accession numbers. We thus reduced the networks and reconstructed condition specific networks corresponding to each expression data by removing all the proteins that are absent and their incident links. Structural organization of these networks was compared by studying topological characteristics. We found that the degree distribution of most of the networks obtained was different from the power law. Additionally, we found changes in clustering coefficient, i.e. local connectivity properties, at two specific time points during the oxidative stress response. Our results suggest the *connectivity of the system* is being modulated as a response to stress or other external stimuli.

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Revealing transcriptional programs in yeast by using 'reporter features' algorithm

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High-throughput analytical techniques for mapping cellular components and quantifying genome-wide molecules have brought new promises and challenges to modern biology. A major challenge resides on how to analyze and extract knowledge from the vast amounts of transcriptome data being generated, in order to elucidate cell transcriptional programs. Commonly used methods for analysis of transcriptome data, such as statistical significance or clustering analyses, implicitly assume that there may be an all-to-all interaction among the genes with similar behavior. However, the dimensionality of the problem can be significantly reduced if we include biological information (e.g., physical and/or functional interactions between bio-molecules) to constrain the interaction space. Based on this concept, we have developed a graph-based method called 'Reporter Features', which allow us to identify key features around which most transcriptional changes occur, by combining network topology and gene expression data. We used four different types of networks available for the yeast *Saccharomyces cerevisiae*– a metabolic network, data on protein-protein interactions, data on protein-DNA interactions and gene ontology annotation list – to determine Reporter Metabolites, Reporter Proteins, Reporter Transcription Factors and Reporter Gene Ontologies, respectively. We used these Reporter Features to analyze large sets of transcriptome data from experiments related with the central carbon metabolism. These analyses offer new insights into the transcriptional programs of glucose repression and Crabtree effect in *S. cerevisiae*.

Yeast temporal coherence

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Temporal coherence of growing organisms depends on complex temporal orchestration. In continuously grown respiring yeast the cellular network self-organises producing temperature-compensated oscillation orchestrated by the cyclical redox state change. These redox cycles can be readily measured by parameters such as dissolved oxygen or NAD(P)H continuously and non-invasively by means of immersed probes. The metabolic and transcriptional organisation of carbon, sulphur and nitrogen incorporation, mitochondrial communication, mRNA turnover and protein turnover are among the top oscillatory components. The organisation of the cell involves the coupling of these transcriptional feedforward mechanisms with proteomic and metabolic feedback control leading to small excitable sub-graphs with a tendency to oscillate, i.e., auto-dynamic. An ensemble of these auto-dynamic network graphs are coupled through metabolic, transcriptional and protein levels to create a self-organising landscape with periods that range between 20 minutes and 50 h. Here we analyse different oscillation frequencies during forced and free-running dynamics, and conclude that a tunable periodic attractor underpins the cellular redox state change that.

Parameter estimation in multi-enzyme systems using time-course analysis and *in situ* techniques

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One of the current challenges in transcriptomics is the analysis of time course experiments. The goal is usually to reveal the genetic interactions involved in the answer to specific cellular environmental changes. At the metabolic level, the analysis of time-course data of enzyme catalized reactions, either obtained from *in situ* kinetic studies or from non-invasive methodologies such as NMR, has also the potential to become a powerful approach in systems biology, both for parameter estimation and metabolic network topology inference.

From an operational point of view, time-course analysis of enzymic reactions and time-course transcriptional data have opposite characteristics: in the former only a few variables are accessible but the time sampling frequency can be made arbitrarily large, whereas in the latter many variables (transcripts) are accessible but only a few time points can be revealed since their number is limited by the high costs of transcriptomic experiments.

In this work we discuss the merits of the association of time-course analysis with *in situ* kinetics from an operational point of view: we indicate how the approach can be streamlined, and what is the improvement in accuracy and cost effectiveness relative to the regression initial value techniques traditionally used in enzymology. The integration of differential evolution optimization algorithm with ODE solvers is being implemented in a software package (AGEDO / S-timator, http://www.dqb.fc.ul.pt/docentes/aferreira/stimator.html) aimed at the time-course analysis of enzyme reaction data. As a case study, we present results on the analysis of methylglyoxal metabolism and MAGE formation in the yeast *Saccharomyces cerevisiae*. Sensytivity analysis using parameters determined by time-course analysis was performed in PLAS (http://www.dqb.fc.ul.pt/docentes/aferreira/plas.html), to identify the most important reactions in methylglyoxal metabolism and to predict glycation phenotypes and flux distributions between catabolic routes.

Unravel the response of yeast glycolysis to temperature changes

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Rapid response to fluctuations in environmental changes is important for competitive fitness and cell survival. A well-studied environmental parameter is temperature variation, which exerts a complex combination of effects on the cell. We have studied the relation of growth rate and temperature of *S. cerevisiae* under well-defined conditions and focused on the effect on glycolysis. We compared chemostat steady state cultures at various temperatures to examine the effects on glycolytic flux. In cultures grown at 38ºC we observed a sevenfold increase in glycolytic flux, which is not accompanied by an equal increase in respiratory rate. We have determined the contribution of distinct cellular regulatory processes that causes this flux increase. We found the changes in metabolic flux through each enzyme are caused by a combination of changes in enzyme concentration, temperature dependent rate changes and changes imposed by the rest of the system at metabolic level. We have extended flux regulation analysis to quantify the contributions of each of these modes of regulation. We will now focus on examples of the various modes of regulation, to understand them in mechanistic detail.

Glycolytic oscillations in two-dimensional arrays of interacting cells

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Synchronisation of glycolytic oscillations in populations of yeast cells has been intensively analysed experimentally (e.g. Richard, P. et al., Eur. J. Biochem. 235 (1996), 238) as well as theoretically (e.g. Wolf, J. and Heinrich, R., Biochem. J. 345 (2000), 321). There is evidence that the individual cells communicate by exchanging products of glycolysis as acetaldehyde, for which the plasma membrane is permeable. In extension to previous models which considered stirred cell suspensions we study oscillations in spatially ordered cells. We aim to reproduce data of experiments where glucose is added to starved cells in a limited region of a cell layer initiating in this way a wave resulting from the propagation of glycolytic oscillations (common project with Thomas Mair et al., University Magdeburg).

For the generation of oscillations in the individual cells a two-component model containing an autocatalytic step is used. Cells are embedded in an extracellular medium in which the added glucose and the extracellular product can diffuse. The model takes into account special kinetic properties of glucose carriers in yeast cells. Intercellular coupling takes place via exchange of the end product. For single cells and a small number of interacting cells, the oscillations can be studied by using bifurcation analysis. Already for three interacting cells with a uniform glucose input, very complex oscillatory states are found. In two-dimensional spatial arrangements of cells where glucose injection is confined to a limited number of cells, waves of glycolytic oscillations are observed which propagate over the whole array. It is shown that the existence of waves depends crucially on the strength of the coupling. If glucose is supplied at two sites in the array, initiated waves annihilate each other.

Measuring the protein concentrations of yeast glycolysis in a high-throughput manner

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Within the project Vertical Genomics, focus is on understanding the control of glycolysis in *Saccharomyces cerevisiae*. This pathway will be studied with respect to promoter activities, mRNA levels, protein concentrations, enzyme activities, metabolite concentrations and metabolic fluxes, under well defined growth conditions. Proteins can be identified by their unique tryptic digestion products (peptides) using mass spectrometry. These peptides can be identified by their measured molecular mass, when analysed with sufficient enough mass accuracy.

To increase detection of peptides from complex mixtures (i.e. a tryptic digest of the soluble fraction of yeast) a reversed phase liquid chromatography (RPLC) system can be coupled directly to a mass spectrometer (MS). The retention time will be used as an extra constraint for identification purposes. Identification based upon LC retention time and accurate mass will only be carried out, after validation by MS/MS to obtain partial sequence info, resulting in an accurate mass and time (AMT) tag. To detect peptides from glycolytic enzymes we use a monolithic PS-DVB column coupled directly to a high performance Fourier transform ion cyclotron resonance mass spectrometer (FTICR)

Differences in protein concentration, from cultures grown under varying experimental conditions, are obtained by comparing MS peak abundancies of glycolytic peptides with peak abundancies from a ^{15}N labeled glycolytic subproteome of a reference culture. The AMT tag method combined with the ^{15}N internal standard approach enables high-throughput analysis of alternations in yeast glycolytic enzyme levels. The data will be correlated with data from other hierarchic levels of the pathway such as the transcriptome, enzyme activities and the metabolome to gain insight in higher order regulation of glycolysis.

Combinatorial regulation of glucose repression in *Saccharomyces cerevisiae*

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The use of combinations of transcription factors in gene regulatory networks greatly enhances the number of possible gene expression patterns, and enables the cell to fine-tune its response to different conditions. This property is believed to be responsible in large part for the complexity of regulatory networks. In the study presented here, combinatorial gene regulation is examined in the context of glucose signaling in *S. cerevisiae.* Besides being an important nutrient, glucose also acts as a signaling molecule in *S. cerevisiae*, affecting a large number of cellular processes, and the expression of a large number of genes. Two major pathways through which glucose signaling is regulated, are the Snf1 *glucose repression pathway* and the Snf3/Rgt2 *glucose induction pathway*. The Snf1 pathway works through Mig1, a transcription factor known to repress gene expression. The glucose induction pathway regulates Mig2, a paralog of Mig1. The DNA binding domains of Mig1 and Mig2 are highly conserved, and they are believed to bind to identical, or similar, DNA sites

The aims of this study are twofold: In addition to identifying the set of target genes that are regulated by Mig1 and Mig2, we also investigated the way in which these transcription factors cooperate in glucose signaling. In this study the expression of all yeast genes was measured in all combinations of knockouts of Mig1 and Mig2 using microarrays. This enabled us to examine the combinatorial aspects behind the gene regulation in a systematic way, and to perform an in-depth analysis of possible binding sites to detect fine differences between the binding specificities of Mig1 and Mig2. About 100 genes were found to be regulated by Mig1 and Mig2. Many known regulatory relationships were recovered, as well as a large number of new ones. Additional evidence that the new genes are indeed regulated by Mig1 and Mig2 came from the presence of consensus binding sites in their promoters, and from examination of an independent gene expression data set obtained by looking at the diauxic shift. Four main modes of regulation by Mig1 and Mig2 were observed: Effect by Mig1 only, partially redundant effect of Mig1 and Mig2, completely redundant effect of Mig1 and Mig2 and effect only by Mig2. Examination of putative binding sites suggests that Mig1 and Mig2 bind to similar sequences. Statistically significant differences between putative Mig1 and Mig2 sites were found only in one position.

Comparative analysis of glucose repression in *Saccharomyces cerevisiae* and *Aspergilli*

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Carbon repression is a global regulatory mechanism in which the presence of glucose or other readily metabolized carbohydrates represses expression of genes involved in the utilization of less-favored carbon sources. Mig1 is the major transcription factor responsible for carbon catabolite repression in *Saccharomyces cerevisiae* and its homologue, CreA is present in *Aspergillus niger,* A. *nidulans* and *A. oryzae.* However, no mechanisms have been identified by which CreA itself is regulated, as well as no components for glucose sensing in aspergilli have been recognized.

Comparative analysis of glucose sensing and repression pathways in *S. cerevisiae* and *A. nidulans*, *A. oryzae* and *A. niger* protein sequences has been performed. Possible homologues to the key components of the two most important pathways involved in glucose repression were identified. The sensors Snf3 and Rgt2 from *S. cerevisiae* seemed to be present in the three fungi, as well as two possible hexose transporters (HXT) in *A. nidulans* (AN4277.3 and AN5860.3) and *A. oryzae* (AO090026000494 and AO090026000259), but only one in *A. niger* (RANI66494). Homologues to the regulatory protein Grr1 (YJR090C) from *S. cerevisiae* were also identified. Nevertheless a homologue of Rgt1, transcription factor of the glucose induction pathway, was not found. Furthermore, the protein phosphatase I (Glc7), involved in regulating the activity of the Snf1-complex in *S. cerevisiae*, was also found in these aspergilli. The analysis pointed at the ORF AN0410.3 in *A. nidulans*, AO090701000351 in *A. oryzae* and RANI68424 in *A. niger*. The bioinformatics approach performed showed that some of the proteins involved in carbon catabolite repression in yeast may also play a role in aspergilli.

Response of *HAP4* gene expression to variations in nutritional conditions

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Saccharomyces cerevisiae has a predominantly fermentative metabolism. When grown on media containing glucose as carbon source, yeast cells repress their respiratory metabolism up to the point where all glucose has been consumed, leaving only ethanol as carbon source. In order to use ethanol, the cell has to reprogram its metabolism, a phase called "diauxic shift" controlled by the HAP complex which is a heteromeric transcriptional complex containing *HAP4*, the activation domain (Buschlen et al., 2003). Expression of *HAP4* is repressed to a low level in the presence of glucose and induced when only non-fermentable carbon sources are available (van Maris *et al*., 2001). The HAP complex is also known to regulate ammonia metabolism and the nitrogen catabolite repression via regulation of the activity of two major enzymes in ammonia metabolism, GDH1 and GDH3 (ter Schure *et al.,* 2000). The expression of *HAP4* varies greatly as a response to limitation or abundance of both carbon and nitrogen in the fermentation medium. In order to investigate the variations in the expression of *HAP4* as a response to nutritional conditions, wild type BY4743 *S. cerevisiae* was grown under nutritional limitations of glucose as the C-source and ammonia as the N-source in both batch and chemostat cultivations. Pulse injections of glucose or $(NH_4)_2SO_4$ were introduced into the cultures to adjust their concentrations in the fermentation media to conditions where the nutrient is not limiting. Concentrations of glucose, ammonia, ethanol, acetic acid, glycerol, pyruvate and acetaldehyde were enzymatically determined. The level of expression of the *HAP4* gene was measured by using real time RT-PCR in samples taken at regular intervals and expression profile of *HAP4* as a response to nutritional variations were obtained. In the carbon limited culture, HAP4 gene was expressed in detectable amounts during the time period when the carbon source glucose was not depleted in the medium. As the concentration of glucose decreased in the medium, the expression level of *HAP4* gene started to increase. As the glucose pulse was injected into the system, the expression levels of *HAP4* declined rapidly as a response of glucose repression. In nitrogen limited cultures, an increase in the expression level of *HAP4* gene was observed when ammonium sulfate pulse injections were introduced into the system.

Structural and functional evolution of the *HAP4* gene, the key regulator of the fermentation/respiration balance in the yeast *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, the HAP transcriptional complex is involved in the fermentation-respiration shift. This complex is composed of 4 subunits. Three subunits are necessary for DNA binding, whereas the Hap4p subunit, glucose-repressed, is the key regulator of the fermentation/respiration balance in yeast *S. cerevisiae*. We previously identified a functional homologue in *Kluyveromyces lactis* which revealed two short conserved motifs and searched these motifs in databases. Putative HAP4 orthologues could then be identified based on these short motifs in ascomycetes. For *Hansenula polymorpha*, which is a widely used yeast for biotechnological processes, two putative *HAP4* homologues containing only the N-terminal conserved domain of the *S. cerevisiae* Hap4 protein were identified. An additional short b-Zip motif, which ressembles the motif found in the *S. cerevisiae* family of YAP proteins is included in the sequence of the second one and also in many HAP4 putative orthologues of other species, provided there are distant from *S. cerevisiae* in the phylogenetic tree.

It was recently found out that *H. polymorpha* can ferment glucose and xylose at 40°C and higher temperatures, which makes this yeast a promising organism for the so called "Simultaneous Saccharification and Fermentation" (SSF) biotechnological process which can be of interest for alcoholic fermentation of lignocellulosic materials. However, the molecular mechanisms that regulate *H. polymorpha* fermentation, are not known. Since molecular genetic tools exist and complete genome sequence is known for this yeast, we have undertaken the study and the characterization of the two putative Hap4p proteins of *H. polymorpha*. We have expressed these putative HpHap4 proteins in *S. cerevisiae* and shown that both are able to restore the growth defect of the *S. cerevisiae hap4* deleted strain. Moreover, the second one confers H_2O_2 resistance to a *S. cerevisiae yap1* mutant hypersensitive to oxidative stress. A set of experiments including global gene expression under control of these two heterologous genes were performed in *S. cerevisiae* to evaluate the functional similarity / diversity of these new genes with Sc*HAP4*. The modular conservation/ insertion of these two motifs (N-domain and b-Zip) in relation with the phylogenetic tree indicates that these domains may have played a crucial role for the evolution of the HAP4 function. The HAP4 regulatory network and its evolution will now be used for modelization studies.

Effect of calorie restriction on oxidative damage in *Saccharomyces cerevisiae* mutant Ubp10: connections among gene silencing, metabolism and aging

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Aging is a complex phenomenon which is connected with several aspects of cellular organization, including chromatin stability, metabolism and intra-cellular oxidative damage. The yeast chromatin deubiquitinating enzyme Ubp10 can be remarkable subject for aging research. In fact, the lack of *UBP10* leads to a complex phenotype, characterized by the derepression of many subtelomeric and stress-related genes, by the accumulation of Reactive Oxygen Species (ROS) and insurgence of typical apoptotic markers in a subpopulation of cells. Interestingly, a strong connection with the life span determinant protein Sir2 has been evidenced: Ubp10p cooperates in maintaining a low level of H3 Lys4 methylation, required for the proper Sir2p association at telomeres. Accumulation and specificity of protein carbonylation as a marker of aging was investigated by framing a panel of carbonylation patterns, obtained by immunodetection of derivatized proteins. Experiments were carried out on W303-1A wild type strain and *ubp10, sir2, ubp10sir2* null mutants, grown in either 2% glucose or Calorie Restriction (CR), a condition known to positively affect yeast life span. The analysis revealed that specific proteins are targeted by oxidation and that CR has a well detectable beneficial effect on the global oxidative damage.

The effect of oxygen level on metabolic flux distribution of *Saccharomyces cerevisiae*

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The metabolic flux responses of the yeast *Saccharomyces cerevisiae* to different oxygen levels were studied by growing the yeast in chemostat conditions under five different oxygen levels, corresponding to 21 (fully aerobic), 2.5, 1.0, 0.5 and 0% of the influx gas of the fermentor. The growth rate was maintained at 0.10 h^{-1}.

Intra-cellular metabolic flux ratios of the central carbon metabolism were determined by ^{13}C tracer experiments. Approximately 10% of the carbon source glucose was replaced by its uniformly ^{13}C labelled variant for 1.5 residence times and the flux ratios were determined from the fine structure of the ^{13}C NMR signals of the proteinogenic amino acids in [^{1}H, ^{13}C] HSQC spectra.

The distribution of metabolic net fluxes was estimated by ^{13}C MFA, i.e. metabolic flux analysis with the flux ratios of the ^{13}C tracer experiments as additional constraints. The metabolic reaction network used in the analysis consists of 25 metabolites and 28 reactions. In addition to the fluxes to the biomass, four extra-cellular fluxes and six intra-cellular flux ratios from the ^{13}C tracer experiments were used in the flux balancing. Due to the additional constraints from the flux ratio analysis, it was not necessary to include the co-factors in the flux balancing.

The result from both the flux ratio analysis and from the ^{13}C-MFA showed that the most dramatic changes in the flux distribution take place at low oxygen levels, while the flux ratios of the 2.5% oxygen cultivation resembled the fully aerobic case. However, the fraction of glucose processed through the pentose phosphate pathway responded to the oxygen level already at high oxygen levels, while the relative anaplerotic flux to the TCA cycle was virtually unaffected until very low oxygen levels are reached.

A vertical genomics approach to the response of yeast catabolism to oxygen

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The Crabtree positive yeast *S. cerevisiae* grows fully respiratorily only when oxygen is saturating and glucose availability is limited, while fully fermentative growth is found under strictly anaerobic conditions. Intermediate situations, characterized by a mixed respiro-fermentative growth, are found either when the oxygen availability is limited, or when well-aerated cultures are subjected to glucose excess. The relative importance of the various regulation mechanisms that lead to the flux distribution over respiratory and fermentative catabolism for a given condition is not known, let alone quantitated.

In this project the contribution of regulatory events to the final catabolic performance is investigated in a time-resolved manner at the metabolic, the enzyme activity, the proteomic and the transcriptomic levels. To this aim, *S. cerevisiae* is cultivated in steady-state glucose-limited chemostat cultures under various, preset and quantitated oxygen availability regimes. The physiological behaviour of these cultures is quantified in terms of specific substrate consumption rates (glucose, O_2) and product formation rates (biomass, CO_2, ethanol etc.) as well as the cellular enzymatic make up and its trascriptome profile. Similar data are being obtained for cultures that are perturbed in their oxygen supply (oxygen upshift or downshift) in order to discriminate cause and effect. The data are being subjected to regulation analysis in order to evaluate to what extent the flux through an enzyme is regulated by metabolic processes (metabolic regulation) and processes affecting the capacity of active enzyme V_{max} (hierarchical regulation).

Gene expression and metabolic analysis of engineered *Saccharomyces cerevisiae* strains for glycerol production

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Saccharomyces cerevisiae is one of the best characterized yeasts and its pathway for glycerol production is well-known. Glycerol is formed by the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate, and the subsequent dephosphorylation of the glycerol-3-phosphate. The yeast cell accumulates glycerol as a response to osmotic stress and to maintain redox balance. Although glycerol is one of the major byproducts of sugar fermentation by yeasts, its maximal production by the species *Saccharomyces cerevisiae* corresponds to only 7 top 10% of the glucose consumed under anaerobic conditions.

In this work, a metabolic engineering strategy was developed to construct an efficient *Saccharomyces cerevisiae* glycerol producer from glucose. *GPD1* encoding glycerol-3-phosphate dehydrogenase and *ALD3* encoding the cytosolic NAD^+-aldehyde dehydrogenase were overexpressed, together with deletion of *TPI1* and *ADH1* encoding, respectively, triose phosphate isomerase and the major NAD^+-dependent alcohol dehydrogenase, in the wild type CEN.PK2 strain. The result of this strategy was an engineered yeast strain, HC42, that exhibited, to the best of our knowledge, the highest yield (0.91 mol /mol glucose) and productivity (3.1 mmol/hr/g dry mass) of glycerol under standard aerated batch condition. However, these genetic interventions led to important metabolic and transcriptomics perturbations in the engineered strain that impede growth and glycerol production efficiency. Among these perturbations was found a hyperaccumulation of intracellular glycerol, that could be partially reduced by overexpression of *FPS1*, and more than 350 genes were differentially expressed. Uisngh MIPS classification, we found that a significant enrichment in categories of genes that belong to metabolism and energy, transport facilitation and protein synthesis. Of particular interest, we noticed upregulation of genes encoding various aldehyde dehydrogenase that may divert part of NADH as well as a strong upregulation of *MIP1* encoding inositol synthase andf *OPI1* encoding a negative regulator of phospholipids synthesis. The overexpression of these latter two genes suggested a partial defect in the inositol synthesis.

Genomic and transcriptomic analysis of lager yeasts

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The genomes of lager yeast arose from the fusion of two yeast strains most closely resembling *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The resultant strains have subsequently undergone genome duplications and rearrangements leading to the generation of a complex aneuploid genome. Recombination between the two parent genomes have led to the presence of mosaic chromosomes.

The complex nature of the genomes of lager yeasts makes transcription analysis difficult. At a first instance, using competitive genomic microarray analysis, we have determined the gene copy number of *S. cerevisiae*-like genes in three lager yeasts. Our analysis reveals the aneuploid nature of the lager yeast chromosomes and pin-points the regions where recombination between homeologous chromosomes has occurred. Sequence analysis in these regions of recombination demonstrates the relatedness of the sequences to *S. cerevisiae* and *S. bayanus* and uncovered novel genes present in the lager yeasts.

The knowledge of the copy number of *S. cerevisiae* and *S. bayanus* genes in the lager yeasts allows us to develop methods to determine the gene expression patterns from the two constituent genomes and to address questions regarding gene dosage in these complex yeasts.

Comparative genomics of species *Saccharomyces*

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Modern genetic taxonomy of the *Saccharomyces* yeasts creates a basis for general taxonomy of yeasts. This genus currently includes six species, viz. *S. cerevisiae, S. bayanus, S. cariocanus, S. kudriavzevii, S. mikatae, S. paradoxus* and hybrid taxon *S. pastorianus*. Species *S. paradoxus* has a worldwide distribution and comprises four geographical populations: European, Far East Asian, North American and Hawaiian (Naumov et al., 1997, 2000). We determined relatedness of recently delimited biological species *S. cariocanus, S. kudriavzevii* and *S. mikatae* and other members of the genus by DNA–DNA reassociation technique, which allows the comparison of the whole genomes of strains under examination. The species *S. bayanus, S. kudriavzevii* and *S. mikatae* are characterized by low DNA homology to one another and to the other species (25–50%). *S. cerevisiae, S. paradoxus* and *S. cariocanus* are the most closely related species in the genus. The nDNA homology values between *S. cerevisiae* and the latter two species are 50%. *S. cariocanus* and *S. paradoxus* exhibited the highest level of DNA similarity – 98% that usually is considered as intraspecific nDNA homology values. The genetic data, however, indicate that four geographical populations of *S. paradoxus* are only partially reproductively isolated from each other, but completely isolated from *S. cariocanus*. Thus, *S. paradoxus* and *S. cariocanus* should be considered separate species. Besides, *S. cariocanus* has a species-specific karyotype clearly distinguishable from that of *S. paradoxus*. *S. paradoxus* strains from different geographical populations have nearly identical karyotype patterns similar to that of *S. cerevisiae*. The karyotype of *S. cariocanus* is characterized by four reciprocal translocations involving eight chromosomes (Naumov et al., 1995, Fischer et al., 2000). Taken together, the genetic and molecular data suggest that *S. paradoxus* and *S. cariocanus* are evolutionary recently diverged sibling species.

Using molecular and genetic analyses, we studied over 100 wild and cultured *S. cerevisiae* strains isolated in various geographical regions. Despite different origins, most of the strains studied yielded fertile hybrids with ascospore viability of 50–100%. In South-East Asia we found a divergent partly genetically isolated population of *S. cerevisiae*. The origin of the *S. cerevisiae* species is discussed.

Comparative genomics of *Saccharomyces* yeasts from red berry and grape winemaking

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During last decades, the production of berry and fruit wines has risen dramatically through the world, especially in the countries with temperate climate. In Russia and Belarus, these wines are produced mainly from apple and black currant must. Using restriction analysis of non-coding rDNA regions, multiplex PCR and molecular karyotyping we examined *Saccharomyces* strains isolated from different berries and various fermentation processes in Russia, Belarus and Ukraine. According to the molecular analysis, most of the strains studied belong to *S. cerevisiae*. There is a correlation between microsatellite fingerprints of strains and the source of their isolation. Strains isolated from juices and from surfaces of different berries showed distinct PCR profiles. In addition to the yeast *S. cerevisiae*, natural interspecific hybrids *S. cerevisiae* x *S. bayanus* var. *uvarum* are documented, for the first time, among isolates from surface of black currant and baker's yeasts. Genome composition of four natural and one laboratory hybrid was analysed. According to genetic and molecular analyses, the hybrids have different genetic constitution. Role of auto- and allopolyploidization in genome evolution of *Saccharomyces* yeasts is discussed.

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Heterozygosity of molecular markers in industrial *Saccharomyces* strains – a new basis for yeast breeding

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Cultured strains of *Saccharomyces cerevisiae* are known to be heterozygotic on some phenotypic physiological properties, viz. fermentation of various sugars, homo-/heterothallism, resistance to killer toxins and copper, etc (Naumov and Zakharov, 1969; Naumov, 1973, 1974; Mortimer et al., 1994). Analysis of wine *S. cerevisiae* strains heterozygosity allowed to describe a phenomenon of homozygotization of monosporic homothallic strains, the so-called "genome renewal" (Mortimer et al., 1994). Homozygotization can take place in both homo- and heterothallic strains through intratetrad mating of ascospores (Zakharov, 1965, 1968, 2005).

We determine a heterozygosity of commercial yeasts on molecular markers, for the first time. According to tetrad analysis, the diploid baker's yeasts studied are heterogenic and heterozygotic on homo/heterothallism. Haploid segregants have been compared on the basis of RAPD-PCR with different primers. RAPD fingerprints were very polymorphic and some bands were lost, compared to fingerprints of the initial yeasts. In contrast, in some segregants additional PCR bands showed up, which, probably, resulted from recombination in heterozygotic DNA. Cluster analysis of the RAPD and RFLP patterns confirmed a pronounced genetic variation among meiotic segregants. Thus, the RAPD and RFLP markers revealed a high heterozygosity of initial yeasts, which can be caused by accumulation of spontaneous mutations. Since industrial strains, usually, do not undergo complete life cycle – ascosporulation, the spontaneous mutations are not eliminated. Mutations accumulated in certain combinations can serve as a source for new genotypes useful in breeding programs with industrial yeasts. Using of heterozygotic molecular markers for purposeful heterosis effect is discussed.

Wine bottling process: microbial assessment and yeast characterisation

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Microbiological stability of the bottled wine is largely dependent on the bottling process. Proliferation of undesired microorganisms leading to product spoilage, can result in significant economical losses for wine producers. Therefore monitoring the microbiota present at the bottling line is of prime importance. In this survey we have monitored the microbial contamination level in a winery bottling line and characterised the yeast biota.

Samples of wine, apparatus surfaces, bottles and corks were collected at different locations during wine bottling. Contamination level was evaluated either directly by membrane filtration or spread plate after convenient serial dilutions, using selective media for total count, yeast, lactic and acetic acid bacteria. After visual differentiation of colony morphotypes, a total of 165 yeast strains representative of each type and location were isolated. Yeast isolates were characterised by restriction enzyme digests of PCR amplified regions of 26S rDNA (1). The 19 different profiles obtained by restriction analysis were compared with those present in our database and for unknown patterns, sequencing of the region D1/D2 of 26S rDNA was performed.

A great diversity of yeast species was found, indicating that these habitats are prone to microbial development. Species belonging to *Zygosaccharomyces* and *Torulaspora* genera were found in the bottling apparatus as well as in the bottled wine. Species belonging to the genera *Cryptococcus* and *Filobasidium* were identified in the corking machine but were not found in the bottled wine, unlike the species *Pichia galeiformis* found in both. The present study allowed evaluating the effectiveness of different sanitation procedures and will lead to the implementation of more adequate practices in the future.

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From the vineyard to the winery: genomic characterization of autochthonous *Saccharomyces cerevisiae* strains

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Traditionally, spontaneous alcoholic fermentation of grape juice into wine is a complex microbial process in which *Saccharomyces cerevisiae* is the predominant yeast species. The use of molecular techniques based on the analysis of DNA polymorphism has also demonstrated that the fermentative process is usually carried out by the sequential and/or concurrent development of different native *S. cerevisiae* strains. Although a high intra-specific diversity is widely recognized, the origin of these yeast strains is rather controversial, the primary source possibly being the winery surfaces and its equipments or the vineyard according to different studies.

In order to enhance knowledge about natural distribution of *S. cerevisiae* strains in the vine-growing and wine-producing habitat, a molecular study was performed in one winery where *Brunello di Montalcino* quality wine is produced without using yeast starters. In particular, 263 *S. cerevisiae* isolates from four ecosystems (the soil of one vineyard, the grapes, the inside surface of a wooden tank and the fermenting must) were genomically characterized using mitochondrial DNA restriction fragment length polymorphism (RFLP-mtDNA) generated by the endonucleases *Rsa*I and *Hinf*I. Among the resulting 30 different mtDNA restriction patterns, 25 corresponded to *S. cerevisiae* strains isolated from one of the four ecosystems taken into consideration. On the contrary, five mtDNA restriction patterns (I, XIV, XV, XVII and XXI) resulted to be common to *S. cerevisiae* isolates from the soil of the vineyard and from the fermenting must, while patterns I and XVII were also showed by isolates from the inside surface of the tank before the vinification. It must be stressed that the pattern I was predominant in the tank as well as during alcoholic fermentation and it was present at significant frequency (30%) in the soil.

These findings demonstrate that must fermenting *S. cerevisiae* strains are closely related to strains occurring in the vineyard and in the winery and suggest that autochthonous yeast strains can be considered as resident populations of a typical wine-producing area.

Application of molecular techniques to fingerprint *Saccharomyces cerevisiae* strains

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In addition to wide-ranging roles in beverage and food processing, *Saccharomyces cerevisiae* plays an important role as a model organism in biochemistry, genetics and molecular biology and in 1996 the complete genome of this yeast species was determined. Afterwards, evidence was provided showing substantial genetic differences among wild *S. cerevisiae* strains. Therefore, exploring the biodiversity of indigenous fermentative strains can be an important contribution towards the understanding and selection of strains with specific phenotypes. In recent years, several methodologies of typing based on DNA polymorphisms have been developed which allowed discrimination among closely related yeast strains. In this work, we report data on the molecular characterization of natural *S. cerevisiae* strains of wine origin. The aim was the individuation of molecular techniques yielding strain-specific profiles useful as markers to recognize *S. cerevisiae* strains during the fermentative process. The wild strains were isolated from spontaneously fermented grapes of *Greco di Basilicata*, a typical grape variety of Basilicata region (Southern Italy), collected in different sampling points in the same vineyard. The strains, identified by molecular techniques as *S. cerevisiae,* were submitted to RAPD-PCR analysis with the primer M13 (GAGGGTGGCGGTTCT) and PCR fingerprinting with microsatellite primer $(GTG)_5$. The strains exhibited different genetic profiles in function of the primer utilized, both primers allowing to discriminate the wild strains. The statistical analysis of the molecular profiles obtained revealed the existence among the strains analyzed of a higher polymorphism with the primer M13 in comparison to the variability obtained with the primer $(GTG)_5$. The strains analyzed during this research did not have contact with wine-cellars, but they represent strains coming from the grapes, i.e. the indigenous population of *S. cerevisiae* present in a vineyard of *Greco di Basilicata*. The genetic diversity found among the strains represents an useful tool for the preservation of the natural yeast biodiversity. Nevertheless, the strain-specific molecular profiles can have a practical application to follow the strain dominance during the fermentative process.

Technological and molecular tools to discriminate a natural population of *Saccharomyces cerevisiae*

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The sensory profile of a wine is characteristic for each vine cultivar and the quality and oenological characteristics of the final wine vary considerably as a function of the strains which performed and/or dominated the fermentation process. Studies have demonstrated that credible proof exists that the vineyard is the primary source for the wine yeasts and that strains naturally present in the grape must may possess some technological traits expressed at desirable level, thus conferring a more significant contribution to the wine. In this perspective the need is for methods which allow to correlate strain characteristics to their residing territory. Here we report results of studies performed on sixty *S. cerevisiae* wild strains, isolated from of three different grape samples collected in the same vineyard. The strains used did not have contact with wine-cellars and represent the dominant *S. cerevisiae* strains of each specific isolation sample. The strains were evaluated for their genetic and technological variability. For the technological characterization, they were screened for the production of secondary compounds in grape must fermentation at lab scale and the amount varied in particular for acetaldehyde, ethyl acetate, isoamyl alcohol and acetic acid. The data analysis by principal components emphasized a certain association between the spatial distribution of the experimental wines and the isolation origin (grape samples) of the strains. Thus, the strains isolated from the sample 1 produced mainly wines characterized by high content of *n*-propanol and acetaldehyde, whereas the strains from the sample 2 yielded wines with elevated amounts of isobutanol and amyl alcohols and those from sample 3 produced wines with low content of ethyl acetate, isobutanol, amyl alcohols and acetic acid. The genetic polymorphism among the sixty *S. cerevisiae* wild strains was evaluated by RAPD-PCR analysis with the primer M13 (GAGGGTGGCGGTTCT). The strains exhibited a significant genetic variability, although they were isolated from the same vineyard, so the isolation area was quite limited. In some cases, a certain correlation was found between the genetic profiles and the isolation origin of the strains (grape samples), emphasizing the influence of the environment on genetic patrimony of yeast population. The individuation of strain biotypes/phenotypes related to the environment represents an useful tool for the traceability of single *S. cerevisiae* strain.

A combined proteomic and transcriptomic approach for cell cycle mutant characterization in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, several pathways are involved to ensure a proper cell cycle progression in response to different environmental as well as intracellular conditions. In this context, a carbon source-modulated critical cell size has to reach to enter S phase at Start, a short interval in late G1 phase after which cells are committed to division. A major role in setting the cell size required for Start execution has been ascribed to the G1 cyclin Cln3. We have recently provided experimental evidence that Cdc28-Cln complex inhibitor Far1 may act as a Cln3 inhibitor in a nutritionally modulated molecular threshold. To gain more insight on molecular mechanism underlying this feature of cell cycle control, we tried to develop an integrated approach allowing to detect differential protein expression pattern as well as to evidence specific transcriptional or translational regulation mechanisms.

Here we show preliminary results obtained by comparison transcriptional profiles as well as protein expression pattern of FAR1 lacking or over expressing mutant and wild type strains, during exponential growth in synthetic medium containing 2% glucose (SCD) or 2% ethanol (SCE). Transcriptional profiles were obtained by using Affimetrix GeneChip technology and DAT file analyses were performed by MAS 5.0 software. Then, mRNA expression values were analysed by using Gene Spring 7.1 and hierarchical clustering were carried out on gene lists by Gene tree algorithm. In parallel, total proteins extracts were separated by 2D-page electrophoresis through a non-linear pH gradient (3–10) in the first dimension and a linear acrylamide gradient (7.5%–16%) in the second dimension. Gel images obtained by Gel code staining and high resolution scanning were compared by PDQuest software to evaluate relative and differential protein expression. Differentially expressed proteins were identified and their identity ascertained by Mass Spectrometry. Finally, for proteins differentially expressed between mutants and wild type, either in SCE or in SCD, protein fold changes were compared to the corresponding mRNA fold changes. Notably, we observed in many cases significant differences between the abundance ratio of mRNA and the corresponding protein, in particular correlation between the two parameters in ethanol was better than in glucose, a condition in which this most of genes were only post transcriptionally modulated. Thus, our preliminary data suggest that our integrated approach can supply useful and not obvious information about molecular mechanisms regulating cell cycle progression. The relevant and possibly universally conserved is not yet understood.

The G_1 to S circuitry in budding yeast

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The cell cycle is one of the complex biological processes that benefit from the systems biology approach. Budding yeast is an established model organism for this study, and several mathematical models have been proposed for its cell cycle[1, 2]. We report a mathematical model of the G_1 to S circuitry that takes into account both the role of Far1[3] and Sic1 cyclin-dependent kinase inhibitors in controlling cell cycle progression and the nucleo/cytoplasmic localization of Sic1 modulated by carbon sources[4]. The model was implemented by a set of ordinary differential equations (ODEs)[5], an approach successfully used to describe the cell cycle control in budding yeast[1, 2] and the cellular response of yeast to hyperosmotic shock[6]. These equations describe the temporal change of the concentrations of the involved proteins and complexes. The model considers the localization of components in different cellular compartments and the cell growth during the G_1 phase, which have been neglected by earlier models. We investigate the dynamics of the G_1/S transition by simulation in several genetic and nutritional set ups and in response to different signaling pathways, extending the model to nutritional modulation of firing of DNA replication origins. The model was found highly consistent with experimental data from literature and from our laboratory.

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Mitotic exit in two dimensions

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Mitosis is initiated in all eukaryotes by activation of cyclin-dependent-kinase (Cdk1) and mitotic cyclin complexes (called Cdc28/Clb2 in budding yeast, *Saccharomyces cerevisiae*). In order to exit from mitosis high Cdk activity needs to be down-regulated. This achieved by activation of Anaphase Promoting Complex (APC) which initiates ubiquitylation dependent degradation of Clb2 mitotic cyclins. APC also ubiquitylates securin, which causes an activation of separase and progression through anaphase of mitosis. Queralt et al. [1] showed recently that separase besides cleaving cohesion molecules between sister chromatids, also inactivates $PP2A^{Cdc55}$ as well, leading to Cdk dependent phosphorylation of Net1. Phosphorylated Net1 releases Cdc14 phosphatase which is required for mitotic exit by down-regulating Cdk activity completely.

Queralt et al. [1] have developed a mathematical model for mitotic exit in budding yeast and they have analyzed the model by computer simulations. They were able to explain all of their experimental situations by numerical simulations of the model. However computer simulations do not provide us with an inside view of the underlying mechanism. Therefore we present here a deeper analysis of the mitotic exit model by using phase-plane technique of dynamical systems theory. By calculating nullclines on the Cdk–Cdc14 phase planes we explain all the experimental results of Queralt et al. [1] including transient Cdc14 release in MEN mutants. We also show why separase overexpression is insufficient to induce mitotic exit in metaphase arrested cells.

[1] Queralt, E., Lehane, C., Novak, B. and Uhlmann, F. (2006) Anaphase-specific Net1 phosphorylation and mitotic exit initiated by separase-dependent down-regulation of $PP2A^{Cdc55}$ phosphatase activity. Cell. (In press)

Identification of modules in a budding yeast cell cycle model

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Complex biological systems can be understood only by the help of mathematical tools. We published a detailed computational model (Chen et al., 2000 *Mol. Biol. Cell* **11**, 369–391) that describes the dynamical properties of budding yeast cell cycle regulation. The Chen model used a set of 9 ordinary differential equations to model the comings and goings of the key cell cycle regulator proteins. Four different Cdk/cyclin complexes were distinguished (Cln3, Cln2, Clb5 and Clb2) and their inhibitors, activators and transcription factors were also incorporated. In the Chen model we used extra equations to integrate the actions of the different Cdk's on bud emergence, DNA replication and mitosis. This model can simulate the behavior of more than 50 mutants. (An updated version (Chen et al., 2004 *Mol. Biol. Cell* **15**, 3841–3862) appeared recently that can match more than 100 mutants.) This very rigorously constructed model can be further investigated with mathematical tools that originally have been developed for analysis of large gas kinetic mechanisms.

Eigenvalue calculations help to understand how the key cell cycle transitions can be connected to autocatalytic loops. Eigenvalues of the Jacobian of the kinetic differential equations tell us if the corresponding system is in excitation (autocatalysis fires) or relaxation phase (approaching a steady state). We found that cell cycle transitions are consequences of autocatalytic explosions of the model.

Sensitivity analysis is widely used to study chemical kinetic models. This tool determines the sensitivities of variables of a differential equation system for parameter changes. In this talk we present how to group the parameters of the cell cycle regulatory network by the similarities of their sensitivity functions. This method introduces a way of systematic modularization of a biological regulatory network. We also present that a change in one parameter can be compensated by a precise modification in a second parameter from the same module and set back the simulation results for the original wild type behavior. Thus our modularization method can predict which parameters and how should be changed to reverse a modification in the cell cycle regulatory system. This could help to find ideal drug targets for cancer treatment to set back the cell cycle system if it collected some undesired modifications.

Modelling the simultaneous growth and death of yeasts growing under optimal and stressing environmental conditions

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Concurrent growth and death of yeast cells under mild stress conditions (temperatures slightly higher than the optimal) were described by N. van Uden and A. Madeira Lopes 25 years ago (*Z. allg. Mikrobiologie*, 10, 515–526. 1970). Some years later, the extensive use of yeasts as models to study cell aging, provided abundant information on the yeast aging process. It was shown that any yeast cell has a limited life span that can oscillate between minutes and about 100 hours, (equivalent to 0 to 40 generations) but not more. Life expectancy seems to depend on the function of a stochastic trigger that sets in motion a programme leading to cell apoptosis and death (S.M. Jazwinsky. *Mol. Microbiol*. 4:337–343. 1990). From these studies it can be concluded that any yeast population has to be formed by growing, apoptotic and dead cells, in different proportions depending on the age of the culture and the environmental conditions. With this knowledge we have developed a mathematical model to analyze and predict the decrease in μ with time due to cell death, based on the stochastic distribution of life spans among the cells.

Moreover, in collaboration with Manuela Corte Real group and in her lab, we have shown that, under hyperosmotic stress conditions, the most relevant mechanism affecting the apparent growth rate is the death of the cells after an apoptotic process (*Mol. Microbiol*. 58:824–834. 2005). Also in this case apoptosis seems to be stochastically triggered in the cells, what introduces a strong heterogeneity in the population that can be observed and measured through flow cytometry. This heterogeneity, stronger as the culture grows old and also with the intensity of the stress factor, is a very important factor to be taken into account in the interpretation of results obtained with yeasts populations under stress. Based on the similarity of mechanisms underlying the decrease of μ in optimal and stressed cultures, we have developed a general stochastic model in which the enhancement of cell death by the stress conditions can be quantified as a function of the amount of stress and the behaviour.

Identification and analysis of controlling genes in yeast metabolism.

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The end goal of building a complete *in silico* model of yeast requires first a coarse grained understanding of the different genes responsible for controlling phenotype. In this study the authors ask the question which of the genes in the yeast metabolic network can be said to exert a governing influence on growth, and more importantly why?

Using large-scale hemizgous mutant competition experiments we have determined which genes cause a significant reduction in growth rate at half dosage. These data can be used as an indication to find the genes that have a significant controlling effect on their respective pathway. This then leads to the question of how do flux control co-efficients in the specific biomass producing pathways overlap with observed haploinsufficiency values for overall cellular growth rate.

Taking two previously modelled yeast pathways showing a significant degree of haploinsufficiency (i.e. the metabolism of sphingolipids and sterols), we apply kinetic modelling and the techniques of metabolic control analysis to determine which of the fluxes in the pathways have a high flux control co-efficient. These predicted values can then be compared to the already observed values for competition experiments, the aim being to determine the relationship between these two sets of values.

A mathematical model for the DNA replication checkpoint in fisson yeast

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Cdc18 is an essential protein in fission yeast because it acts as a licensing factor for DNA replication. Recent experiments from Paul Nurse's lab (D. Hermand and P. Nurse, unpublished) suggest that Cdc18 is also involved in DNA replication checkpoint mechanism which inhibits cell cycle progression through Tyr-phosphorylation of Cdk/cyclin (both Cdc2/Cig2 and Cdc2/Cdc13) complexes. Here we present a mathematical model for the regulation of Cdc2/Cig2 Tyr-phosphorylation and for the role of Cdc18 in DNA replication checkpoint. In the model Cdc2/Cig2 kinase activity is regulated by transcriptional control, by reversible Tyr-phosphorylation, by stoichiometric inhibitor (Rum1) binding and by ubiquitin mediated proteolysis. Here we propose that the primary Tyr-kinase phosphorylating Cdc2/Cig2 is Mik1. Cdc2/Cig2 kinase promotes Rum1, Mik1 and Cdc18 degradation and inhibits its own transcription factor. Cdc18 level is regulated by transcriptional control and by Cdk phosphorylation dependent proteolysis. Cdc18 protein can exist in free and chromatin associated forms and all the forms of Cdc18 activate the DNA replication checkpoint, although with different efficiency. The checkpoint mechanism works through inhibition of Tyr-phosphatases dephosphorylating Cdk/cyclin complexes.

The Cig2 and the Cdc18 dynamics describe the mechanism that drives periodic S phases during endoreplication cycles in the absence of Cdc13. By supplementing this mechanism with the regulation of Cdc2/Cdc13 kinase we can describe the alternating S and M phases during mitotic cell cycles.

Blocking DNA replication stabilizes chromatin bound Cdc18 and the DNA replication checkpoint gets activated. This inhibits both Cdc2/Cdc13 and Cdc2/Cig2 dephosphorylation therefore the transcription factor responsible for Mik1, Cdc18 and Cig2 stays active and the level of these proteins stays high. After releasing the DNA replication block Cdc2/Cig2 gets rapidly dephosphorylated and activated thereby turning off transcription factor and promoting Mik1, Cdc18 degradation. Cdc18 removal eliminates the DNA replication checkpoint. The model can explain not only wild type behavior, but many other situations as well (e.g. *cdc18*^{OP} and *cdc18TA* mutants), and it suggests a few yet untested predictions.

Incorporating protein biosynthesis into the S*accharomyces cerevisiae* genome scale metabolic model

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Based on the stoichiometry of biochemical reactions that occur into the cell, genome-scale metabolic models can be used to quantify metabolic fluxes, which are regarded as the final representation of the physiological state of the cell. For *Saccharomyces cerevisiae,* the genome-scale model has been constructed considering the metabolic reactions from mitochondria and cytosol; the set of reactions comprised in the central carbon and biosynthetic pathways. This model does not account for protein synthesis, which consumes the majority of total energy produced by a rapidly growing cell. To include protein synthesis in the model, we surveyed the available literature, and hereby it was possible to identify enzymatic reactions and gene functions involved in transcription and translation: mRNA transcription, mRNA processing, mRNA export out of the nucleus, translation initiation, translation elongation, translation termination, translation elongation, and mRNA decay. We have incorporated these new "reactions" into the genome-scale model for *S. cerevisiae* to obtain the first set of *in silico* experiments that take place that include protein biosynthesis.

Identification of operational constraints that shape the response of yeast to heat shock

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Understanding the relationship between gene expression changes, enzyme activity shifts, and the corresponding physiological adaptive response of organisms to environmental cues is crucial in explaining how cells cope with stress. For example, adaptation of yeast to heat shock involves a characteristic profile of changes to the expression levels of genes coding for enzymes of the glycolytic pathway and some of its branches. The determination of changes in gene expression profiles provides a descriptive picture of the adaptive response to stress. However, it does not explain why a particular profile is selected for any given response. We used mathematical models and analysis of *in silico* gene expression profiles (GEPs) to understand how changes in gene expression correlate to an efficient response of yeast cells to heat shock. An exhaustive set of GEPs, matched with the corresponding set of enzyme activities, was simulated and analyzed. The effectiveness of each profile in the response to heat shock was evaluated according to relevant physiological and functional criteria. The small subset of GEPs that lead to effective physiological responses after heat shock was identified as the result of the tuning of several evolutionary criteria. The experimentally observed transcriptional changes in response to heat shock belong to this set and can be explained by quantitative design principles at the physiological level that ultimately constrain changes in gene expression. Our theoretical approach suggests a method for understanding the combined effect of changes in the expression of multiple genes on the activity of metabolic pathways, and consequently on the adaptation of cellular metabolism to heat shock. This method identifies quantitative design principles that rfacilitate understating the response of the cell to stress.

MAGE in yeast: a cellular model of protein glycation *in vivo*

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Protein glycation by methylglyoxal is a non-enzymatic pos-translational modification where arginine and lysine side-chains form a chemically diversified group of MAGE (methylglyoxal-derived advanced glycation end-products). MAGE are the hallmark of diabetes and amyloidotic neurodegenerative diseases. Since methylglyoxal is produced non-enzymatically during glycolysis, its formation occurs in all cells. We discovered the ocurrence of protein glycation in *Saccharomyces cerevisiae* and identified different glycation phenotypes. This validates yeast as a model to investigate protein glycation, its effects and cellular responses *in vivo*. Using MALDI-TOF, we identified enolase as the primary glycation target in *S. cerevisiae*. Two other glycolytic enzymes are also glycated, aldolase and phosphoglycerate mutase. The refolding chaperone pathway, comprising Hsp26 and Hsp71 is also activated under glycation conditions. Remarkably, despite a dose dependent activity loss upon glycation, glycolytic flux and D-glycerol metabolism remain unchanged.

To understand why yeast is so resilient to glycation, a detailed glycolytic model was built, including methylglyoxal metabolism, using experimentally determined parameters. The software package PLAS was used for the numerical integration of the differential equations and to perform a sensitivity analysis (http://www.dqb.fc.ul.pt/docentes/aferreira/plas.html). None of the glycated enzymes has any effects on glycolytic flux. To obtain measurable flux changes, enolase activity would have to decrease to 5% of its reference value. Even a simultaneous 30% activity loss of the glycated enzymes would only cause a 0.02% decrease of glycolytic flux. Despite its non-enzymatic origin, methylglyoxal concentration is sensitive to D-glycerol dehydrogenase and triose phosphate isomerase activity. Its steady-state concentration also depends on the synergistic action of glyoxalase I and aldose reductase activities, the main catabolic routes for this 2-oxoaldehyde. Yeast cells endured a selective pressure towards an efficient use of D-glucose, with high methylglyoxal formation as a side effect. Glycation is a fact of life for these cells, and it mainly affects some glycolytic enzymes. These enzymes might be deployed to contain methylglyoxal that evades its enzymatic catabolism, therefore protecting other cellular components.

The cycling of trehalose in *S. cerevisiae*, a mathematical model

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In the post-omics era, all the compounds that suffice to constitute a living organism will be known. Ultimately, the ambition is to understand the functioning of the living organism from the properties of these compounds. As in many other fields of science, the large scale of complex data sets calls for their mathematical analysis and computer stimulation. Mathematical modeling is now taking a center stage in biology, and its use and importance is likely to increase. It has in particular been shown that the modeling of biochemical pathways can contribute significantly to our understanding of metabolic regulation networks, and, as a consequence, improve the efficiency of metabolic engineering approaches. In order to alter metabolic fluxes within a living system, it is necessary to understand where the control point (key reactions) of a specific pathway lies. Also, the conditions in a living cell might differ drastically from those in a test tube environment. Regulation of the activity of enzymes by metabolites produced elsewhere in cell metabolism may be overlooked. One of the main focus points of our group's research is to change the flux of a fermenting yeast cell towards higher accumulation of reserve carbohydrates and a subsequent decrease in ethanol concentration. Here we present a mathematical model that describes the behaviour of the enzymes involved in the metabolism of trehalose, linked to the kinetic model on yeast glycolysis (Teusink et al. 2000) based on known and experimental determined kinetic data. In this version of the model a competitive inhibition by T6P on hexosekinase is included, which acts as a stabilizer on glycoliytic influx, thus allowing the model to describe the delta *tps1* phenotype successfully.

Identification of an intracellular signalling complex for Ras/cAMP pathway in yeast: experimental evidences and modelling

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Preliminary data obtained in our laboratory show that the Ras2 protein accumulates in the GTP-bound form in a strain deleted for the gene encoding adenylate cyclase (*cyr1, pde2, msn2, msn4*). This result is in agreement with data in literature suggesting the Ira and Cyr1 proteins might be involved in the formation of a multi protein complex which would give them the possibility to interact with the Ras proteins, to stimulate their intrinsic GTPase activity and consequently to turn off the signal.

New experiments done with strains expressing different deletion mutants of Cyr1 protein indicate that the defects in Ras2-GTP regulation are due to the absence of the Ras binding domains of the Cyr1 protein, since mutants missing only the N-terminal region of the protein and strains *fil*1 and *lcr*1 showed a normal basal and glucose-induced levels of Ras2-GTP/total Ras2, while in mutants missing one of the two Ras binding sites mapped on adenylate cyclase the Ras2 protein accumulates mostly in the GTP-bound form, like in the strain deleted for the gene encoding adenylate cyclase.

In addition we present evidences that most of the component involved in this signalling pathway (Cdc25, Cyr1, Ira1, Ras2) localises on internal yeast membranes and not on the plasma membrane as thought before, suggesting the presence of a large signalling complex inside the yeast cells.

Moreover we started to develop a new system biology approach by modelling the Ras/cAMP/PKA pathway of yeast using a P-system approach that allow to construct and simulate a signalling network taking in account the compartmentalisation of the different component.

Dynamic modelling of thiamine regulation in *Saccharomyces cerevisiae* based on High Performance Liquid Chromatography (HPLC) measurements

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Thiamine pyrophosphate (ThPP) also known as vitamin B1, is the active form of thiamine (Th) and plays an essential role in cellular metabolism. Regulation of thiamine is a complex dynamic process involving different mechanisms such as transport, de novo synthesis, and gene regulation. For the well studied model system Saccharomyces cerevisiae the different cellular components that take part in the thiamine regulation process are relatively well known. This information is sufficient to setup an a priori static network diagram, also called model network structure, of the process. Such a structure maps the components (e.g., genes, mRNA, proteins, metabolites) and the interactions (e.g., transcription, translation, reactions) between these components within a considered system. However, such a static network structure is not sufficient to gain an increased understanding of the dynamic properties of the thiamine regulation process and to predict the behaviour of the system in the presence of perturbations or modifications. In order to gain such an increased understanding a dynamic model of the regulation system is needed, and can be obtained by assigning reasonable mathematical rate expressions and corresponding parameters to the rate variables with the static model. The process of dynamic modelling is iterative and might lead to a revision of the initial static network structure and the mathematical rate expressions. In this work we consider dynamic modelling of thiamine regulation in Saccharomyces cerevisiae. In a first step we limit ourselves to a smaller subsystem, namely to the extra and intra cellular thiamine (de)phosphorylation and thiamine transport into the cell.

Our approach to the modelling of thiamine regulation is based on the selection of reasonable rate expressions, relying on biochemical insight into the system and mathematical analysis. Estimation of the numeric values of the involved parameters is done based on quantitative time series measurements of thiamine, thiamine monophosphate (ThP), and thiamine pyrophosphate obtained by High Performance Liquid Chromatography (HPLC).

Results:

1. An a priori static model network structure of the complete thiamine regulation system.
2. Quantitative time series measurements of the concentrations of Th, ThP, ThPP in cell extracts from yeast cell cultures submitted to different environmental conditions (with and w/o thiamine, glucose, etc.) where obtained experimentally. These measurements showed an accumulation of Th, instead of ThPP, in contrast to what was believed earlier.
3. A first dynamic model for the subsystem involving extra and intra cellular thiamine (de)phosphorylation and thiamine transport into the cell.

Fatty acid profile modulation of the lipids accumulated by *Rhodotorula glutinis*

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In a context of petroleum resources dwindling, the renewable carbon exploitation for the production of biomolecules, substitutes for oil in energetic or chemical roads, represents an important stake. Fatty acids are precursory molecules of chemical or enzymatic reactions for producing compounds with valuable function depending on their number of carbon and their insaturation degree. Microbiological production of fatty acids, through an accumulation of triacylglycerols, from carbohydrate substrates, constitutes an alternative to the usual route (vegetal oil). This way has high advantages such as the control of the fatty acid profile by the strain and the culture operating parameters and the diversification of the agricultural product valorisation.

The aim of our work consists in the quantification of the triacylglycerol accumulation mechanisms of the yeast *Rhodotorula glutinis* in order to maximize the triacylglycerol intracellular productivity and the substrate to lipid conversion yield, and to modulate the fatty acid composition with the operating culture conditions.

The strategy combines simulation and experiments. From previous works mentioned in the literature, a kinetic model is elaborated to simulate the evolution of the yeast growth, the lipid production, the substrate consumption versus time. Fed-batch cultures of *Rhodotorula glutinis,* on synthetic medium and glucose, at different temperatures and pH 5.5, are carried out. The dynamical behavior of the yeast is quantified in order to understand the yeast metabolism involved during the induction phase and the lipid accumulation. 132 $g.L^{-1}$ of dry biomass, 53% (w/w) of lipid cell content with a conversion yield value of 0.20 $g_{Lip}.g_{Glu}^{-1}$ are performed in 70 hours at 30°C. The lipid profile accumulated by the yeast, at different temperatures, are compared. Different strategies are tested to induce the lipid accumulation. Theoretical and experimental results are presented and discussed.

Monitoring of transcript regulation and protein production related stress responses in *Pichia pastoris* secreting Fab antibody fragments

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The rapid transcriptional profiling method VTT-TRAC has been applied to monitor the levels of a subset of mRNAs coding for UPR-regulated and stress-connected genes in chemostat cultivations of a *P. pastoris* strain secreting the 2F5 antibody fragment. Specific marker genes have been chosen to deliver insights into the general physiological status of the cells under production conditions (including growth, protein synthesis, oxygen and nutrient limitation responses) with the main focus on secretion stress connected genes (UPR, ERAD, posttranslational processing). TRAC analysis of shake flask cultivations of *P. pastoris* strains co-overexpressing *S. cerevisiae* UPR-transcription factor Hac1p was applied to identify UPR-targets in *P. pastoris*. Consequently, the induction of UPR-target genes due to heterologous protein production could be shown for the first time in *P. pastoris*. Overexpression of *S. cerevisiae* protein disulfide isomerase (PDI1) could not diminish UPR burden. Most of the investigated genes seem to be unaffected by PDI1 overexpression at a transcriptional level, although 2F5 Fab secretion rates are enhanced under these conditions.

As product formation is known to be strongly dependent on cultivation conditions, the influence of different cultivation temperatures has been analysed. Transcript formation rates of the two respective product genes (for Fab light chain and heavy chain mRNA) have been set in correlation to the mRNA levels of folding related genes such as KAR2 and PDI1, and additionally to the specific product formation rate of secreted Fab. Interestingly, although the transcriptional levels of our product genes were reduced at lower temperature, specific productivity of the 2F5 Fab protein was significantly increased. Thus it is tempting to speculate that at lower temperature a reduced amount of folding stress is imposed on the cells, consequently leading to a higher rate of correctly folded product. Also the chaperon KAR2/BiP, which is commonly seen as a marker of unfolded protein stress appeared among the genes downregulated at lower temperature, while the transcription of genes coding for elements of the vesicular transport system is enhanced. Additionally, the levels of intracellularly retained antibody fragments and the UPR marker protein BiP (Kar2) were analyzed by immunofluorescence and flow cytometry. Conclusions will be drawn regarding the regulation patterns of the analyzed marker genes during the adaptation to different growth conditions, and their connection to product formation and secretion will be discussed.

Heterologous expression of isotypically labelled *Trichoderma reesei* tyrosinase 2 in *Pichia pastoris*

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Tyrosinase (EC 1.14.18.1) is a copper-containing oxidase that is widely distributed in mammals, invertebrates, plants and microorganisms. In mammals the enzyme is essential for the formation of melanin pigments, whereas tyrosinases in fruit and vegetables are related to the browning reaction that occurs upon bruising and long term storage. Tyrosinase is of great interest for many applications in the field of medicine, biotechnology and food engineering. It is a promising target enzyme for prodrug activations in melanomas and in biotechnological applications including crosslinking of protein matrices. It is of great importance to find ligands and inhibitors for tyrosinase. Structural studies and screening for ligands and inhibitors can be carried out using NMR spectroscopy with isotopically labeled tyrosinase. Therefore, we cloned a novel tyrosinase from *Trichoderma reesei* and expressed it heterologously in the methylotrophic yeast *Pichia pastoris*.

A novel tyrosinase, tyrosinase 2 (TYR2), was cloned from *Trichoderma reesei*. The cDNA sequence was expressed under the control of the AOX1 promoter in the *Pichia pastoris* X-33 strain. The Saccharomyces cerevisiae alpha-MF prepro sequence was used for secretion and an N-terminal His6-tag was fused to the tyrosinase to facilitate the detection and purification of the recombinant protein. Heterologous expression was carried out in shake flask cultivations and the enzymatic activity was measured directly on the culture medium, using L-Dopa as a substrate. Extensive optimisation of the expression in shake flasks was carried out as the stable isotope labels are costly. Different temperatures, different CuSO4 and NH4SO4 concentrations and different shake flasks were tested. The expression level of recombinant TYR2 was increased tenfold as a result of the optimisation. Metabolic 15N-labeling of TYR2 was carried out with 15NH4SO4 in minimal medium to assess its suitability for investigations by NMR spectroscopy. Initial 3D heteronuclear 1H-15N HSQC NMR spectrum of TYR2 showed signals with chemical shifts typical of folded proteins.

The *Trichoderma reesei* tyrosinase 2 was successfully expressed and uniformly 15N-labeled in the yeast *Pichia pastoris*. This methylotrophic yeast is a suitable expression system for the production of recombinant proteins for NMR studies as it is cost-effective and possesses the ability to perform many of the posttranslational modifications of higher eukaryotes.

Transcriptome analysis of the unfolded protein response pathway in the methylotrophic yeast *Hansenula polymorpha*

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Accumulation of misfolded protein in the endoplasmic reticulum induces unfolded protein response (UPR) which brings about multiple cellular protective events to ensure proper protein folding and secretion. We investigated the global changes of gene expression induced by UPR in the methylotrophic yeast *Hansenula polymorpha*, a promising host for the production of recombinant proteins. Whole-genome cDNA microarrays of *H. polymorpha* were used to identify UPR target genes after the treatment of tunicamycin and DTT to induce UPR by disrupting protein folding and modification. The genes up-regulated in response to secretion stress included a large number of protein secretion related genes, which can be categorized into secretion (translocation, vesicle transport), processing (folding, disulfide bond exchange, glycosylation), and quality control (ERAD pathway), as reported in the traditional yeast *Saccharomyces cerevisiae*. Comparison of the gene expression profiles was also carried out between the wild-type strain and the Hphac1 null mutant strain, in which the *H. polymorpha* homolog of the *S. cerevisiae HAC1* gene, encoding the key UPR transcription factor, had been deleted. Genes of which the expression levels were significantly changed in the Hphac1 null mutant strain were categorized as targets of the HAC1-mediated signaling pathway in *H. polymorpha*. Our transcriptome analysis data revealed several interesting differences in transcriptional responses to secretion stress between two different yeast species *H. polymorpha* and *S. cerevisiae*.

Engineering of the *Pichia pastoris* glycosylation systems: homogenous glycosylation and human-type complex N-glycans

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Pichia pastoris is widely used as an expression host for heterologous proteins. Its N-glycosylation synthesis pathway mirrors that of typical mammalian cells up to the point where $Man_8GlcNAc_2$ N-glycosylated proteins exit the endoplasmic reticulum (ER). In contrast to higher eukaryotes Pichia N-glycans can be elongated to a hyperglycosylated carbohydrate with an alpha-1,6-backbone carrying up to 40 mannose residues. Since these heterogeneous structures are potentially immunogenic, hamper downstream processing of the heterologous protein and result in rapid clearance from the blood stream, we have engineered the Pichia N-glycan synthesis pathway to allow the synthesis of hybrid, more human-like oligosaccharides. In a first step, the OCH1 gene, encoding the transferase initiating hypermannosylation, is inactivated. In combination with the overexpression of an ER-retained alpha-1,2-mannosidase, originally cloned from Trichoderma reesei, this strategy results in the homogenous synthesis of $Man_5GlcNAc_2$. Further conversion to a biantennary N-glycan ($GlcNAc_2Man_3GlcNAc_2$) was obtained via Golgi targeting of mammalian GlcNAc-transferase I and II as well as Mannosidase II. Galactosylation was acquired by expression of a fusion construct between the S. pombe UDP-Glucose-4-epimerase and the human β-1,4-galactosyltransferase I, targeted to the Golgi apparatus.

ABSTRACTS OF
POSTER SESSION II
Posters P56 – P108

Monte carlo sampling, data interpretation, and control analysis of yeast metabolic pathways

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Designing an effective Metabolic Engineering strategy necessitates a systems-level investigation on the linkage between genotypic mutations and phenotypic responses. This linkage is yet to be fully discovered due to the uncertainty in physiochemical parameters and the complicated interactions between cellular metabolism and its bioprocess environment. We have developed a computational and statistical framework which is based on Metabolic Control Analysis and uses a Monte Carlo sampling method to simulate the uncertainty in the values of the system parameters. In this study, we generalize this framework to incorporate the central cellular processes, such as cell growth, and different bioprocess conditions, such as different types of bioreactors. This allows a realistic understanding of cellular events by examining them in the context of industrial growth behavior.

This framework is applied on the compartmentalized central carbon pathways of *Saccharomyces cerevisiae* metabolism considering the growth environment of batch and chemostat reactor and integrating information from metabolic flux analysis. Statistical analysis of the large-scale sampling results indicates that yeast cells growing in batch culture condition exhibit dramatically different control schemes from those growing in a chemostat. The difference is mainly due to the feedback introduced by the constraints of the chemostat. The control of the enzymes on the rates of the substrate uptake, product excretion, and cell growth and its practical implication are discussed. Clustering of the reaction steps according to the similarity of their responses to enzyme activity perturbations reveals functional coupling of metabolic reactions.

Reverse engineering algorithm to recover gene regulatory network with very limited number of gene expression data: bayesian orthogonal least squares (bols) algorithm

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The development of efficient computational methods to elucidate gene regulatory networks using gene expression data is one of the great challenges in systems biology. There is proven evidence that linear systems of ordinary differential equations can be used to model gene regulatory systems. Reverse engineering using linear systems is an underdetermined and an ill-conditioned problem, i.e. the amount of microarray measurements is limited due to the cost of experiments and the solution of the linear system is sensitive to noise in datasets. Thus, networks with large numbers of genes/proteins/components will likely require stronger optimization algorithms. We present an efficient algorithm for reverse engineering gene regulatory networks from microarray datasets using a linear system of ordinary differential equations. We combined orthogonal least square method, second order derivative for network pruning, and Bayesian model comparison to reverse-engineer gene regulatory networks. The algorithm can be used to reconstruct partial network structure with an extremely small number of data points. The method was successfully applied to predict networks and to interpret yeast cell cycle gene expression data.

Systems and synthetic biology of yeast metabolism

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Metabolism is one of the key cellular processes providing necessary precursor molecules and free energy necessary for biosynthesis and maintenance. This central role of metabolism is evident by two facts, firstly, several of the metabolic pathways are well conserved across different domains of life; and secondly, cellular response to genetic and environmental perturbations is often reflected and/or mediated through changes in the metabolism. Consequently, it is not surprising that several diseases (e.g. diabetes, cancer, obesity) are closely associated with metabolic disorders/malfunctioning. Moreover, metabolism of yeast is largely used as "cell factory" for producing variety of industrial chemicals and pharmaceutical products. Thus understanding organizational and functional principles of yeast metabolic networks is an essential pre-requisite for designing rational strategies, not only for combating diseases, but also for metabolic engineering of "cell factories".

Owing to the topological and regulatory complexity of metabolism, emergent systemic properties play an equally important role in the operation of metabolism, as the properties of its constituting components. The poster will be centered on understanding the biological logic behind large-scale organization, operation and design of yeast metabolism from systems perspective. Since cellular metabolism, as reflected in the metabolite levels and fluxes, is an integrated result of mass balance constraints and regulation, metabolic operation can also be broadly classified into, stoichiometric (and topological) analysis and regulation of metabolism. Stoichiometry represents the mass balance constraints on metabolic network at (pseudo-) steady state and can be viewed as limits on the possible operational modes in *n*-dimensional flux space. Regulatory networks impose additional constraints on this solution space and thus together these two decide what metabolic phenotype will be observed under given conditions.

Kinetic constraints for formation of steady states in biochemical networks

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The constraint-based analysis has emerged as a useful tool for analysis of biochemical networks. This work introduces the concept of kinetic constraints. It is shown that maximal reaction rates are appropriate constraints only for isolated enzymatic reactions. For biochemical networks, it is revealed that constraints for formation of a steady state require specific relationships between maximal reaction rates of all enzymes. The constraints for a branched network are significantly different from those for a cyclic network. Moreover, the constraints do not require Michaelis-Menten constants for most enzymes, and they only require the constants for the enzymes at the branching or cyclic point. Reversibility of reactions at system boundary or branching point may significantly impact on kinetic constraints. When enzymes are regulated, regulations may impose severe kinetic constraints for the formation of steady states. As the complexity of a network increases, kinetic constraints become more severe. In addition, it is demonstrated that kinetic constraints for networks with co-regulation can be analysed using the approach. In general, co-regulation enhances the constraints and therefore larger fluctuations in fluxes can be accommodated in the networks with co-regulation. As a first example of the application, we derive the kinetic constraints for an actual network that describes sucrose accumulation in the sugar cane culm, and confirm their validity using numerical simulations.

Metabolic temperature compensation of metabolic flux in yeast *Saccharomyces cerevisiae*

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Temperature dependence of all biochemical reactions is described by the exponential (the Arrhenius) equation. However, some integral biological processes on the acclimation time-scale show non-exponential relationship with temperature variation. We distinguish two-level hierarchical compensatory organization in the metabolic system: immediate (local) and global. Local compensation is an intrinsic feature of the topology of metabolic pathway architecture and it implies that there must be steps in the pathway with negative control coefficients in order to achieve compensation of a certain flux towards a certain metabolite:

$$\frac{d \ln \nu_j}{d \ln T} = \frac{1}{RT} \sum_{i=1}^{N} C_i^{\nu_j} E_i = 0$$

Such compensation is achieved through adjusting the local activation energies (E_i) of the component processes through different molecular mechanisms, like allosteric regulation, phosphorilation and etc (E-compensation),

i.e. by processes that lasted on the minute of faster scale, therefore this compensation type works against fast and irregular temperature variation as temperature fluctuations are. From the other side, global compensation allows involvement of slow regulatory mechanisms as gene regulation, meaning switching on/off pathways, adjusting synthesis/degradation rates and etc which changes topology of the metabolic network and flux control coefficients ($C_i^{\nu_j}$) of some component processes in order to optimize the metabolism to new temperature conditions (C-compensation) facilitating seasonal acclimation. Compensation of catabolism is one of the requirements to control the homeostasis of the energy metabolism, i.e. constancy of the energy (EC) and catabolic reduction (CRC) charges. In reality the E- and C-compensations are taking place simultaneously. DNA microarray experiment shown that under long-term low temperature (15°C) acclimation yeast are likely switching to anaerobic metabolism even in the presence of the oxygen, which is very likely due to the loss of aerobic capacity by mitochondria. In the same time cytoplasmatic protein synthesis is highly activated, which points to the quantitative compensation of loss of molecular functions. Additional anaerobic experiments with long-term yeast acclimation to different temperatures have shown that temperature profile of the general metabolic activity (measured as mCal/min/mg of wet biomass using DSC) reduces its overall activation energy (E_J) with acclimation.

$$E_J = \sum_{i=1}^{N} C_i^{\nu_j} E_i$$

Therefore change of the E_J indicate metabolic rearrangements that likely compensate the temperature effect.

Atom-resolved analysis of metabolic pathways

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In metabolic pathways, enzymes rearrange molecular structure of substrates into products. Analysis of tracer study using isotopes for estimating metabolic flux requires the formal description of molecular recombination patterns including structural symmetry and chirality. To computationally assist the analysis, we present a software system, Atomic Reconstruction of Metabolism (ARM) to explore and visualize logical traces of arbitrary metabolic pathways in a defined network. The software program uses atomic correspondence between metabolites for more than 2000 biochemical reactions whose coenzymes are identified. Each reaction is decomposed into a set of "substructural correspondences" between metabolites, and the positional traces for all carbon and nitrogen atoms are resolved. By combining these correspondences, users can search metabolic pathways between any two compounds. The system has been recently expanded to include sterol and fatty acid metabolism. The advantage of the system is that it can substitute abstract description such as 'phosphatidyl group' with concretes such as fatty acids in the analysis. Indeed, hundreds of molecular species are represented by general names such as phosphatidyl serine or choline, and it is impossible to manually trace all recombination patterns in their complex network.

Another update of the system is the integration of metabolic maps into the pathway search software. The metabolic maps in PostScript format can be uploaded to the software and all texts (corresponding to compound names or enzyme names) in the picture are automatically associated with entries in the atom-resolved database. With this function, users can trace pathways on user-defined metabolic maps and grasp its metabolic capability without referencing shredded pieces of metabolic pathways in books or traditional databases. The system is available online from http://www.metabolome.jp/.

A framework for direct metabolic flux estimation

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We present FluxDirect, a novel computational framework for metabolic flux estimation from incomplete isotopomer data. In FluxDirect isotopomer measurements are first propagated in the metabolic network to obtain new constraints to the isotopomer distributions of metabolites in the network. The efficient propagation is facilitated by a fragment flow analysis of a metabolic network [1]. The method can utilize any measurement information produced by common (tandem) MS- and NMR-techniques [3].

After the propagation step the basic linear stoichiometric equation system bounding the fluxes is augmented with linear measurement constraints derived from the propagated isotopomer information. If the rank of the augmented linear equation system is full, the fluxes can be easily solved. If the rank of an equation system is less than full we can output upper and lower bounds for the fluxes. The general stability of an augmented equation system can be investigated by computing the condition number of the system. Unlike in most existing methods for flux estimation, techniques of nonlinear optimization are not required. Thus FluxDirect escapes the danger of convergence to local optima.

Fragment flow analysis techniques introduced for efficient propagation of isotopomer measurement data also facilitate the experimental planning of isotopomer tracer experiments. As the development of measurement techniques for internal metabolites can be tedious, it is worthwhile to look for a small set of metabolites giving adequate flux information. We have shown that such small sets can be found for FluxDirect by applying computational set cover methods [2]. The flux estimation framework is empirically tested with a model of central carbon metabolism of *S. cerevisiae*.

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^{13}C-constrained metabolic flux balancing analysis of *Pichia pastoris* grown on different carbon sources

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^{13}C-constrained flux balancing analysis based on NMR data [1] has been applied as a method for the estimation of intracellular carbon fluxes of Pichia pastoris cells. In this approach, the underdetermined system of metabolite balances deduced from stoichiometric relations and measured extracellular rates is complemented with ^{13}C constraints from metabolic flux ratio analysis [2, 3]. Steady state fluxes in central carbon metabolism of the methylotrophic yeast grown in chemostat cultures were estimated by ^{13}C-constrained flux balancing method in different ^{13}C-labeled carbon source experiments. In particular, cells were grown aerobically in chemostat cultures fed at two dilution rates (0.05 h^{-1}, 0.16 h^{-1}) with glucose, glycerol or varying mixtures of glycerol and methanol as carbon sources.

In previous studies [4, 5], the metabolic pathways associated with the TCA cycle intermediates of P. pastoris were characterised in terms of metabolic flux ratios based on the ^{13}C labelling pattern of proteinogenic amino acids using ^{13}C-NMR measurements. Based on this data, analysis of metabolic flux responses to environmental modifications (carbon source and growth rate) has now been performed by ^{13}C-constrained flux balancing analysis. A metabolic network has been reconstructed from literature data and summarized in a stoichiometric model including up to 54 internal metabolic reactions distributed into mitochondrial, cytoplasmic and biomass generation reactions, which include up to 55 metabolites. The underdetermined linear equation system was solved using flux partitioning ratios as additional constraints to obtain the global net flux solution. Overall, calculations show that the co-assimilation of methanol has a clear impact on the distribution of metabolic fluxes through the pentose phosphate pathway (PPP) and TCA cycle, as well as the distribution of methanol carbon into assimilatory and dissimilatory (direct oxidation to CO_2) pathways. Also, such impact is clearly dependant on the specific growth rate of the cells. This study brings new insights on the P. pastoris' methanol metabolism complex regulation in the presence of a second carbon source, revealing important implications for biotechnological applications.

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Metabolic high-throughput characterization of yeast deletion mutants

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This work aims at the in-depth metabolic investigation of deletion mutants of the eukaryotic model organism *S. cerevisiae.* We developed and applied new stoichiometric and flux profiling methods in combination with bioinformatic analysis with the aim of predicting the function of specific genes and their regulatory mechanisms as well as to study environmental influences. A new cultivation method with integrated oxygen sensing in 96-well microtiter plates combined with metabolite balancing and ^{13}C-labelling analysis using MALDI-TOF mass spectrometric measurement allows determination of major pathway activities, e.g. glycolysis, pentose phosphate pathway (PPP), TCA-cycle, respiratory chain, and anaplerotic reactions using glucose and galactose as substates.

From the cultivations specific growth rate, biomass yield on carbon substrate and oxygen and ethanol yield on carbon substrate could be precisely determine. Maximum specific growth rates were in the same range for both sugars. On glucose the growth was predominantly fermentative with high yield of ethanol, low yield of biomass and low oxygen consumption rate. On galactose respiration was more active with correspondingly lower ethanol yields, higher biomass yields and higher rates of oxygen consumption. Several strains showed unexpectedly high growth rate, rates of ethanol production and respiration as compared to the wild type. Overall these parameters determined for each mutant allowed statistically significant discrimination of all mutants studied. A new MALDI-TOF method allowed highly parallel analysis of ^{13}C labelling of ethanol after enzymatic oxidation to acetaldehyde and simultaneous reaction with 2,4-dinitrophenylhydrazine to form 2,4-acetaldehyde dinitrophenylhydrazone that can be directly analyzed by MALDI-TOF-MS. In experiments with feeding of 1-^{13}C-glucose or 1-^{13}C-galactose it is then possible to determine the flux partitioning between glycolysis and PPP and the ethanol concentration in one run applying 2-^{13}C-ethanol as internal standard. Based on this measurement and metabolite balancing whole flux maps were identified. Estimated flux partitioning rates were comparable to reported values from continuous culture (Frick and Wittmann, 2005). One observed tendency was a general positive correlation between biomass yield and flux partitioning into PPP. The method provides a sound basis for quantitative metabolic characterization of yeast deletion mutants on a large scale.

Noninvasive analysis of metabolic intermediates in complex biological probes

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Quantitative data analysis of metabolic pathways is often hampered by the high complexity of the biological probe, which requires excessive probe treatment when invasive techniques are used. Ideally, noninvasive methods should provide quantitative data sets with high temporal and spatial resolution without the need to perturb the biological system. Such noninvasive methods are commonly based on radiation related techniques, e.g. light. We demonstrate that infrared spectroscopy, in conjunction with chemometric analysis, is a powerful tool for the investigation of metabolic pathways in complex biological probes. As a model system we have measured the kinetics of glycolytic sugar degradation in a yeast extract. Most of the glycolytic intermediates can be determined. Even the complexation of the adenine nucleotides with magnesium could be followed, thereby demonstrating the importance of the magnesium salt of ATP as a substrate for the glycolytic enzymes. Moreover, when glycolytic oscillations are induced, we find different shapes of the amplitudes for the different intermediates. Such a behavior is also known from chemical oscillators, as for example the Belousov-Zhabotinsky reaction, but not for glycolysis. The possibility to include an infrared microscope into the setup, also allows to extending this analysis for spatiotemporal phenomena, as for example traveling reaction diffusion waves.

Revised protocols for quantitative yeast metabolites extraction. Application to a glucose pulse to carbon-limited yeast cultures, which reveals a transient activation of the purine salvage pathway

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Quantitative extraction and accurate measurement of intracellular metabolites are two conditions that must be strictly overcome for quantitative physiological studies. Here, we revised our original procedure of yeast metabolites extraction and show that (i) less than 5% of metabolites leakage takes place at the quenching step, and (ii) with a few exception, the stability of intermediates of central metabolic pathways are not altered during the boiling step in a buffered ethanol solution. However, a loss of spiked metabolites ranging from 5 to 30% depending on the type of metabolites was attributed to their adsorption on cellular debris after ethanol treatment. We also set up simplification of methodologies for quantitative metabolites measurement by high performance ionic chromatography (HPIC) techniques with emphasis for purines and pyrimidines bases using a variable wavelength detector set at 220 and 260 nm in tandem with suppressed conductivity detector for their identification and quantification. These protocols were successfully applied to the study of a glucose pulse to carbon-limited yeast cultures. This study underscored a glucose-induced activation of the purine salvage pathway, as indicated by a transient drop of ATP and ADP and a concomitant transient rise of IMP and inosine. This metabolic perturbation could be in part accounted for by a rapid and reversible activation of the IMP-5'-nucleotidase encoded by *ISN1*. This mechanism was specifically induced by rapid fermentescible sugars but not by galactose nor by osmotic pressure. Mutants of the purine metabolic pathways are currently under investigation to quantify the importance of each of the branches in the formation and reassimilation of inosine.

Variability of biofilm formation in different strains of *Saccharomyces cerevisiae*

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Among the different genes that regulate biofilm formation in strains of *Saccharomyces cerevisiae, FLO11* plays a fundamental role. In a previous work we showed that *FLO11* is required for air-liquid interfacial biofilm and we proposed a model for biofilm formation based on the ability of cells to entrap carbon dioxide, providing buoyancy. *FLO11* belongs to the adhesin gene family, including *FLO1*, *FLO5*, *FLO9* and *FLO10* and the protein encoded by each gene has considerable sequence identity with other family members. Other authors showed that the gene size of *FLO1* and the number of intragenic repeat sequences creates quantitative alteration in adhesion, flocculation and biofilm formation phenotypes. In this work, in order to observe correlations between *FLO11* gene size and cell adhesion, we analysed several Sardinian biofilm forming strains of *Saccharomyces cerevisiae* (flor strains) with different genetic backgrounds. We amplified *FLO11* and its intragenic repeat sequences in all the analysed strains and we correlated the lengths with the following phenotypes: biofilm formation, adhesion to plastic, mat formation and idrophobicity. Interestingly, we observed a variable correlation between *FLO11* gene size and the described phenotypes. In particular, different strains showed differences in adhesion to plastic and biofilm formation or idrophobicity and biofilm formation independently of the expansion and contraction in *FLO11* lenght.

Biofilms formation of yeasts belonging to different genus on a new media

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In many environments microrganisms exist predominantly in biofilms. Concerning yeasts, *Candida* species remain the more studied yeast for this biofilm formation cause of medical impacts. Paradoxically, little is known about yeast belonging to other genus. Eladium is a new polysaccharide produced by *Rhizobium* spp. and owning the ability to replace agar. It offers differents advantages like to let microorganisms growing faster than on agar medium. Using this new solidified substance of media culture, we have tested the ability of wild yeast strains belonging to different genus to develop biofilms. We have observed that some of them have this ability whereas others are maladjusted to develop biofilms. We have also studied the effects of glucose and nitrogen concentrations on this biofilm formation.

The pyruvate decarboxylase activity assay of yeast *in situ*

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Cytoplasmic pyruvate decarboxylase EC 4.1.1.1 (PDC), is one of the key yeast enzymes which allows precise and recurrent description of fermentative metabolism. PDC is the first enzyme of glycolytic pathway which, under anaerobic conditions, leads to nonoxidative decarboxylation of pyruvate with acetaldehyde as end product. However, this enzyme is also capable to condensate of acetaldehyde and pyruvate and to form acetoine. There is also a known fact, that some of yeast strains could produce acetate from accumulated acetaldehyde. Therefore, in the case of enzymatic reactions *in situ*, control of all products of these metabolic pathways, in which PDC could participate, is necessary. The aim of this research was to develop a suitable method of yeast pyruvate decarboxylase activity assay *in situ* and to compare enzyme activity of yeast cells in different physiological states.

Yeast *Saccharomyces cerevisiae* Bc16a – distillery strain was grown under aerated conditions on an orbital shaker in such nutrient media as YPM and wort broth with 1% and 12% maltose respectively and in fermentative medium with 12% maltose. Enzymatic assay was conducted in cell suspension with digitonin, as permabilisation agent, and sodium pyruvate, as a substrate, at temperature 30°C. PDC metabolites – acetaldehyde, ethanol, acetoine and acetate were detected using GC technique with Headspace Autosampler. Different parameters: type and concentration of substrate, minimal effective concentration of digitonin, cell density, reaction time and effect of pyrazole, as alcohol dehydrogenase inhibitor, were checked in order to optimize PDC assay *in situ*.

In the concentration range of yeast cells from 1×10^7 to 1×10^8/ml the linear correlation was noticed between produced acetaldehyde and cell density. For the given conditions, in the presence of 0,05 M pyruvate and 0,05% digitonin, the enzymatic reaction was linear up to 20. minutes of assay. During incubation there was no formation of ethanol and therefore pyrazole wasn't necessary for the assay. PDC activity in *S. cerevisiae* cells depends on physiological state of cells and culture conditions (oxygen availability, sugar concentration). The maximal PDC activity of yeast cells was observed on the 3rd day of fermentation process, whereas, on 7th day of aerobic cultivation, PDC activity was not seen.

Adaptive evolution of a recombinant lactose-consuming *Saccharomyces cerevisiae* strain

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In previous work, a recombinant *S. cerevisiae* flocculent strain (NCYC869-A3/T1, or simply T1) with the ability to express both the *LAC4* (coding for beta-galactosidase) and *LAC12* (lactose permease) genes of *Kluyveromyces lactis* was constructed (Domingues *et al.*, Appl Microbiol Biotechnol 51:621–626, 1999). The original recombinant obtained (T1) was able to metabolise lactose but slowly. Thus, it was subjected to an adaptation period, where the recombinant yeast was kept in liquid lactose medium, refreshed periodically. Cells collected after the adaptation process presented improved fermentative characteristics compared to the original transformant, namely higher growth rate and higher ethanol productivity. This evolved strain was named T1-E. The fermentative parameters (shake-flask cultivations with buffered lactose defined mineral medium) of strain T1-E are similar to *K. lactis* wild-type strain CBS2359 (NRRL-Y1140).

We aim at elucidating what happened during the process of adaptation/evolution that the yeast went through. The plasmid used for transformation (pKR1B-Lac4-1), which harbors a 13 kb region of the *K. lactis* genome including *LAC4* and *LAC12* genes, remained autonomous in the recombinant strain. Plasmid isolated from T1 (before adaptation) was identical to pKR1B-Lac4-1. However, we found that the plasmid isolated from T1-E carries a 1594 bp deletion (positions -518 to -2111 from the 5' end of *LAC4*) in the promoter region between *LAC4* and *LAC12* genes. This deletion may have improved the transcription of one or both of the genes, which may be the cause for the improved lactose consumption phenotype of the evolved strain. In lactose cultivations, the intracellular beta-galactosidase activity of strain T1-E is about 40 times higher when compared to T1. Moreover, the level of beta-galactosidase activity in strain T1-E is comparable to *K. lactis* CBS2359.

Microarray analysis showed increased expression of genes related with transposable elements in T1-E compared to T1, which reflects the selective pressure that the yeast suffered during the adaptation process. The transcriptome (*S. cerevisiae*) analysis did not revealed other important differences between T1 and T1-E.

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Correlation between mannoprotein biosynthesis and fermentative behavior in *Saccharomyces cerevisiae* wine strains

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Currently genetic improvement and selection of wine yeast starter strains are carried out by considering strain characteristics related mainly to fermentative and biotechnological attitude and only partially to stress resistance ability. However stressful conditions associated with must fermentation can result in decreased viability and growth rates in the more sensitive *S. cerevisiae* strains, and thus to stuck or sluggish fermentations. The aim of this work was to understand which genetic features a wine yeast strain needs to possess to resist stuck and sluggish fermentation. To this end, we have analyzed the differences in the transcriptome of two *S. cerevisiae* wine strains, characterized by a different fermentative behaviour, during stress fermentations. The experimental design allowed us to evaluate the influences of the different genetic background and to consider only the differences due to the adaptation to the stress conditions. The results obtained showed common and specific responses. In particular we observed differences in the expression of genes related to glucose metabolism (HXT and ADH genes) and to mannoprotein biosynthesis (*TIR1*, *TIR3* and *TIR4*). Considering the importance of mannoproteins not only in conferring wine organoleptic quality but also in cell adaptation to hypoxic conditions, the results obtained could lead to identify a correlation between these cellular components and strain fermentative behaviour during winemaking.

Effect of GABA assimilation on *Saccharomyces cerevisiae* central carbon metabolism

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The gamma-aminobutyrate (GABA) shunt, found in a wide range of organisms, is an alternative route for the conversion of glutamate into succinate, which involves the enzymes glutamate decarboxylase, GABA amino-transferase and succinate semi-aldehyde dehydrogenase, encoded in *S. cerevisiae* by *GAD1*, *UGA1* and *UGA2* respectively[1]. Genome wide expression analyses during wine fermentation have shown that these genes are up-regulated during the nitrogen-limited stationary phase[2].

To investigate the potential roles of this pathway in glutamate catabolism and GABA assimilation, a functional analysis was carried out in the strain V5 during wine fermentation. Whereas glutamate is catabolized through this pathway at a very low rate, external GABA can be efficiently converted to succinate via Uga2p and Uga1p, with a yield up to 0.8 mol/mol.

To evaluate the effect of GABA catabolism on yeast central metabolism, we analysed the transcriptome and metabolite profiles of V5 during wine fermentation. In the presence of 4 g/L GABA, the expression of all the genes involved in GABA transport and catabolism was increased. In addition, significant changes were observed in the expression of genes involved in glutamate metabolism and carboxylic acids formation. Assimilation of GABA caused increased formation of alpha-ketoglutarate (KG) and hydroxyglutarate, which was consistent with up-regulation of *PYC1, IDH1* and *GDH2* and down-regulation of *GDH1*. This indicates that the cells compensate for the lack of KG, that is consumed in the GABA transamination reaction, by a fine adjustment of the amount of enzymes involved in its formation and utilisation. In addition, the concentration of the main fermentative by-products was modified, which probably reflects the need to balance the excess of NAD(P)H generated through the Uga2p reaction. This was not coupled to transcriptional changes, suggesting that these modifications mainly result from allosteric control. Analysis of the global effects of GABA on the metabolic network is currently underway.

[1] Coleman et al. (2001) *J Biol Chem*. 20:244–250.

[2] Rossignol et al. (2003) *Yeast*, 20:1369–1385.

Physiological characterization of active dry wine yeast rehydration under oenological conditions

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Yeasts are commonly used in winemaking as "Active Dry Wine Yeast" (ADWY), which are "ready-to-use" after a rehydration period (30 minutes) in warm water (37 ºC). This short rehydration period is essential to optimal recovering of the cell activity and the population size, leading to a shortening of the lag phase and a better control of fermentation kinetics and performance. Physiological stability and "fitness" of ADWY cultures relate to the maintenance of cell viability and vitality during the process of yeast manufacturing, including desiccation and storage.

We have studied the biochemical and biophysical behaviour of AWDY during its rehydration process under oenological conditions. Scanning electron microscopy technique was used to determine the water influx into the cell, by measuring yeast cell size. We found that the whole pool of cells recovers their volume during the first 15 minutes of rehydration. Leakage of intracellular compounds (OD 260 nm) was found to increase through rehydration process. However, not changes in membrane permeability were found, in fact the maximal permeability to violet crystal was found 3 minutes before rehydration started. Intracellular/extracellular trehalose contents were also determined all along rehydration process. Three tested ADWY samples showed the expected high levels contents of intracellular trehalose (ranking from 14.54% to 18.68% dry weight) that partially solubilised. Viability of yeast cells was measured by plating and live/dead cells percentage was determined by fluorescence microscopy. Additionally, we tested several rehydration media as a way to improve yeast early vitality and adaptation to the must.

Influx of water into the cell is fast and Rehydration is a traumatic process for the cells, mainly affecting yeast viability. We conclude that enriched rehydration media with presence of nutrients (Synthetic must diluted to reach 0.5 g/l of sugars) improve de vitality of yeast after rehydration.

Physiological characterization of *Saccharomyces cerevisiae* during wine fermentation

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During wine making yeasts are subjected to an extremely wide gradient of stress situations. Grape must represents the first stress with a very high sugar concentration, low pH, low levels of other nutrients and antimicrobial agents, like copper, sulphur and pesticides. As fermentation proceeds, sugar concentration decreases and ethanol concentration increases, pH decreases and temperature rises enhancing the deleterious effect of the other forms of stress. Although very many studies have been performed on the physiology of *S. cerevisiae* almost nothing is known on how the cells behave under the stress conditions of winemaking in real must. This situation leads to the fact that it is often difficult to extrapolate from these studies useful interpretations to explain yeast performance in the winery.

Our main objective was to perform an integrated analysis of the performance of *S. cerevisiae* while producing wine in order to improve the wine production as far as it depends on yeast fermentation. A *S. cerevisiae* strain was selected from a commercial starter, based on its fermentative capacity, and used to inoculate white grape must in all the experimental assays in the project. Must was centrifuged and filter sterilized. Anaerobic small reactors were designed and the fermentation conditions settled (agitation, sampling, inoculation) and standardized for all the work. Four different growth situations were selected: the beginning and the middle of exponential growth phase, the beginning of stationary phase and the end of the must fermentation, when no sugar was detected by standard methods. We compared with the same strain grown under the common lab conditions (mineral medium, 2% glucose).

The results obtained indicated a similar evolution between OD, dry weight and number of viable cells. Trehalose and glycogen content during growth varied as well as cell leakness and H^+-ATPase activity, presenting their maximum value at mid exponential phase. Fermentative and respiratory capacities, sugar transport, ATP content, heat sensitivity have been measured. Glucose and fructose consumption measured by 13C NMR and cytoplasm and vacuole pH, polyphosphate content and sugar phosphate metabolites levels measured by 31P NMR were evaluated. Results will be discussed under an oenological perspective. C.P. is Post-doc fellowship (SFRH/BPD/20263/2004) funded by FCT, Portugal.

Survey of *Saccharomyces cerevisiae* populations during red wine fermentation

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The increased use of pure selected cultures (active dry yeast-ADY) required the development of new identification methods at the strain level to evaluate the success of the inoculation process. Several methods enabling the differentiation of *S. cerevisiae* strains, namely, RAPD, karyotype analysis, mitochondrial DNA restriction analysis and microsatellite genotyping have been described (1, 2). In the present work we have evaluated *S. cerevisiae* population dynamics during red wine vinifications with different commercial products and the addition of ADY by analysis of six microsatellite loci.

Samples were collected after 36 h (T1) and 84 h (T2) of fermentation and a number of 30 colonies were isolated from each experimental condition at each sampling time. Microsatellite (SSR) amplifications were performed using two multiplex reactions, and for each loci, the size and number of alleles as well as the number of genotypes were determined. Different discriminatory power was observed for the 6 microsatellite markers used. The results enabled to verify the predominance of the inoculated strain, although almost 20% of the isolates corresponded to indigenous strains. Among these a great diversity of genotypes was found. For some of the additives tested an increase in the number of indigenous *S. cerevisiae* strains was found at time T2, indicating a possible positive influence on the survival of these strains. SSR markers proved to be a reliable technique to differentiate at strain level, being an appropriate tool for monitoring and controlling wine fermentations.

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Analysis of genes of *S. cerevisiae* involved in thermotolerance during wine fermentations at low temperature

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Low temperatures (10–15ºC) are used in wine fermentations to enhance both production and better maintenance of volatile flavours. Thus, white and rosé wines with greater aromatic complexity should be produced at low temperatures. However, temperature affects both the yeast growth and the fermentation rate, therefore low temperatures produces longer and sluggish fermentations. In order to understand the biochemical and molecular mechanisms underlying the adaptation of the wine yeast at low temperatures, we recently carried out a global transcriptomic analysis of a wine yeast strain during grape must fermentation at low and optimum temperature. This study revealed a large number of genes with different transcriptional activity between the two fermentation temperatures.

In the present study our objective is to confirm the importance of some of those genes in yeast cold adaptation. A total of ten genes (*CSF1*, *HSP12*, *HSP26*, *HSP104*, *LTE1*, *LOT2*, *NSR1*, *TCP1*, *TIP1* and *TIR2*), involved in stress response, ribosomal formation and nutrient transport, were selected for a subsequent transcriptional analysis throughout wine fermentation by real-time PCR. This comprehensive transcriptomic analysis in the different stages of wine fermentation and at different temperatures showed a regulation differential for those genes. *NSR1* and *LOT2* were highly expressed during the first hours of fermentation and repressed during the stationary phase. Conversely *HSP12* and *HSP26* were activated at the entrance of cells into stationary phase. *TIP1* was only activated at the final stages of fermentation. *LTE1* showed a high activity throughout the wine fermentation. Lastly, *CSF1* and *HSP104* were the only genes whose expression was higher at low temperature in all the time-points analysed. We are currently constructing mutated and overexpressed strains of those genes in a lab strain, and in a commercial wine strain. Both, growth rate and fermentation activity of those strains were tested at low (13ºC) and optimum (25ºC) temperature. In agreement with transcriptomic analysis, *Δcsf1* and *Δlte1* strain showed a growth-deficiency at low temperature while were hardly affected at 25ºC.

A better understanding of the molecular mechanism of adaptation at low temperature fermentation may improve in the future the performance of this wine yeast either by genetic modification or by adaptation during industrial production.

Vertical genomics: adaptation of respiring cells to anaerobic sugar-excess conditions

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The primary role of bakers' yeast (*Saccharomyces cerevisiae*) in the leavening of bread dough is the production of carbon dioxide via the alcoholic fermentation of sugars. Within this process an important parameter will be fermentative capacity, defined as the specific rate of carbon dioxide (and ethanol) production immediately upon introduction of yeast into dough. However, alcoholic fermentation is highly undesirable during the industrial production of bakers' yeast, as it reduces the biomass yield on the carbohydrate feedstock. Industrial bakers' yeast production is therefore, performed in aerobic, sugar-limited fed-batch cultures. Hence, conditions during production of bakers' yeast differ drastically from the dough environment, which is anaerobic and initially contains an excess of sugars. The goal of this project is to understand and ultimately find ways to efficiently control the regulatory mechanisms (from gene to flux) that govern the induction of fermentative capacity. This project is part of the overall program "Vertical Genomics", which is a collaboration between six research groups from different universities (TU delft, UvA and VU).

The dynamic control of fermentative capacity and activity will be simulated and analysed in detail. Aerobic glucose-limited chemostat culture will be used to simulate the yeast production process. Chemostat cultures are preferred over fed-batches, as the culture conditions can be defined and tightly controlled. The dynamic dough environment is simulated by shifting the chemostat to anaerobic batch conditions, followed by a glucose pulse. To understand at which cellular 'level' the fermentative capacity, and therefore glycolysis, is regulated (i.e. transcriptional or post-transcriptional control) the experimental approach includes analysis at various levels, i.e. transcript level (micro-array/ qPCR), protein levels (protein chips/ MS), enzyme activity (enzyme assays) and in vivo carbon-fluxes (stoichiometric modelling).

High gravity fermentations for low calorie beer production

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Today there is an increasing interest in the use of high gravity fermentation in brewing. High-gravity fermentation produces beer wort of 18° Plato or higher and results in beer with more consistent quality. In order to get an increased understanding of the influence of high gravity fermentation on yeast physiology, we established model fermentation conditions where the sugar uptake and product formation were studied in detail. We compared a standard fermentation at 14° Plato and fermentations at 21° Plato. The wort at 21° Plato was prepared by adding highly fermentable maltose and glucose syrups to the standard wort. In both cases the lager beer yeast strain Weihenstephan 34/70 was used and maximum growth was observed after 48 hours of fermentation. The levels of free amino nitrogen (FAN) gradually decreased in the first 48 hours of the fermentations whereafter it stayed around 50 mg/L until the end of the fermentations and then reached a plateau until the end of the fermentations. Characterization of the carbohydrate profile was determined by HPLC and HPAEC. In the 21° Plato fermentations, the initial total sugar concentration reached 150 g/L and the fermentations ended with some residual amounts of maltose and maltotriose and final ethanol concentrations of 55–56 g/L. However, higher viability and vitality of the yeast cells was observed in fermentations with added maltose syrup. As flavour and aroma profiles may depend on gravity, we studied changes in the formation of secondary metabolites as well. The samples for the analytical aroma characterisation were extracted using the solid phase microextraction technique and analysed using GC-MS.

Monitoring gene expression by TRAC through very high gravity brewing fermentations

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Brewers' yeast is subjected to constantly changing environmental conditions during wort fermentation, such as decreasing levels of carbon, nitrogen and oxygen and increasing level of ethanol. Cells can rapidly adapt to changing surroundings by transcriptional regulation. Changes in the genomic expression can indicate the physiological state and condition of yeast in the brewing process. Both conventional and genome-wide transcription analyses have been used to study gene expression during laboratory and industrial scale wort fermentations, but for logistic and economic reasons, data have been collected from only a limited number of samples. Here we have applied to very high gravity brewing fermentations a novel transcriptional analysis method called TRAC (TRanscript analysis with aid of Affinity Capture) for frequent expression analysis of a focused gene set. TRAC enables multiplex mRNA target analysis simultaneously from a large number of samples in a cost- and time-efficient manner. We have selected more than 70 marker genes that code for proteins involved in various pathways relevant to the brewing process, such as maltose, glycerol and lipid metabolism, glucose fermentation, amino acid biosynthesis, aroma formation and flocculation and measured their expression at frequencies up to 2 h.

Results showed rapid changes in gene expression during the first hours of fermentations for several genes. For instance genes involved in maltose metabolism (*MALx3*), glucose fermentation (*HXK2, ADH1,3,4*), ergosterol (*ERG3,13*) and glycerol synthesis (*GPD1*) were strongly up-regulated between 2 to 6 h after pitching. Several genes showed two or more well defined peaks during the first 24 h. Some (not all) glucose-repressible genes (*ADH2, HXK1, GLK1*) were down-regulated as expected during early fermentation while glucose was present, and up-regulation was observed after 20 h of fermentation. By the time (72 h) yeast growth had stopped and total sugars had dropped by about 50%, most selected genes showed low expression and total mRNA was less than half the levels during growth. However, relatively high transcript levels were found, e.g., for some genes involved in flocculation (*FLO8, FLO11*) and glucose phosphorylation on a non-fermentable carbon source (*GLK1*) during the final fermentation stages (120–200 h).

The metabolic profiles of immobilized brewery yeast

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Immobilization of microbial cells is increasingly applied in biotechnological processes. The main benefits can be seen in improved quality of biotechnologies, linked with such traditional fermentation processes as production of beer and further in open technological prospects to novel biotechnologies. Brewing industry is deeply interested, for economical reason, in brewery yeast cells immobilization and its application to continuous processes. It is expected to see in immobilized cell technology the advantage of the reducing time of processes without reduction of product quality. The use of immobilized yeasts may be interesting alternative for conventional processes with increasing productivity of cells loading in bioreactors.

The leading idea of this study was to use method of yeast cells entrapment inside special foamed alginate cores with internal multichambers and to investigate of cells growth in the state of immobilization. We were also interested in evaluation of their ability to conduct ethanol fermentation with specific metabolite profiles, important for proper taste and flavour.

Different *Saccharomyces cerevisiae* brewery, bottom-fermenting yeast strains were used. The primary fermentations were conducted in bath cultures in 12^{o}P in malt wort at temperature 10°C during 7 days with free and immobilized yeast strains. The analysis of carbohydrates was carried out by HPLC – high-performance liquid chromatography. The flavor and aroma compounds (ethanol, higher alcohols and esters) was controlled by GC – gas chromatography analysis with Headspace Autosampler.

The results of studies have shown high ethanol production by entrapped yeast cells and proper profile of green beer flavor. The production of higher alcohols and esters by free and immobilized cells was comparable, but amount of acetaldehyde produced by immobilized yeast was significantly decreased. For each tested brewery yeast strain a specific metabolic profiles were seen. Sequential passages of encapsulated yeast cells into fresh fermentation media have shown the significant reduction of time required for primary fermentation.

Adhesion of selected yeast strains on solid type of carriers

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Cell adhesion is the fundamental phenomenon that govern and describes bioengineering processes that employ cell immobilization with focusing on different biotechnological applications, including beer and biofuel production. The microtopography of the contact surface, physiological state of cells, their phase of growth and nutrient availability are important in determining the adhesion process.

Specific objective of our research was to study how immobilisation conditions: type of culture medium, cell density, physiological state of yeasts and incubation time affected cells attachment to the different solid carriers – three types of hydroxylapatite and chamotte tablets. The industrial brewery and distillery strains *Saccharomyces cerevisiae* and unconventional amylolytic strain *Debaryomyces occidentalis* from different physiological states were used in this study. Microbial adhesion was conducted in nutrient and minimal culture media. In all the experiments, for the estimation of the number of adhered cells and effectiveness of immobilization, microscopic method and methylene blue staining were applied and, in some cases, additionally, DAPI fluorymetric method was also employed.

The immobilization of selected yeasts on hydroxylapatie carriers was weak. However, when incubation of cells was conducted under starvation condition, the scale of observed cell immobilization after 24 hours was higher, especially in the case of *D. occidentalis* strain. The significant differences between cell density and the rate of adhesion were not observed for all tested yeast cells. Therefore we postulate that adhesion on hydroxylapatite carriers has only reversible character. The better results we observed in the case of chamotte – the number of immobilized cells was about $10^6 \div 10^7$ cell per carrier and cell adhesion was stable during 72 hours of fermentation. However, formation of three-dimensional cell structure and microcolonies were not observed. Therefore we can conclude, that the optimal adhesion conditions should be estimated individually for each yeast strain, solid carrier and fermentation process. Determination of the most preferable conditions of adhesion will allow for efficient application of immobilized yeast cells for beer production and conversion of starch to ethanol.

Characteristic of crabtree-negative yeast *Debaryomyces occidentalis* encapsulated in foamed alginate beads

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Immobilization of whole cells by entrapment method is a simple technique widely used in research and industrial applications. Such biotechnological approach has many advantages due to better operational stability and higher efficiency of biocatalysis. Although starch offers a high-yielding ethanol source, yeast *Saccharomyces cerevisiae* with superior ethanol fermentation capability is unable to convert starch into ethanol due to lack of complex of amylolytic enzymes. Contrary to its abilities, unconventional yeast *Debaryomyces occidentalis,* described by Ingledev as a „super yeast", secretes both α-amylase and glucoamylase, which together result in a complete degradation of starch into fermentable glucose. Because of these enzymes irreversible inactivation at 60°C and pH optimum between 5 to 6, they are ideally suited for commercial bioethanol or low ethanol beer production. However, the barrier in wide application of *D. occidentalis* in continuous fermentation processes is seen in fact that this respiratory, Crabtree-negative yeast requires a growth-limiting supply of oxygen to secrete amylases and to conduct alcoholic fermentation.

In the present work was developed new, unique method of yeast immobilization and its utilization in specific biotechnological processes. The alginate multichamber cores with yeast cells were formed in traditional way from foamed basic solutions. The research was focused on the determination of growth patterns, cell viability and metabolism of respiratory yeast cells encapsulated inside alginate cores. The application of the novel immobilization technique for *D. occidentalis* resulted in a high viability of entrapped cells. The initial cell concentration in the core was 10^4 cfu and after period of adaptation during cultivation of the cores in the growth medium increased to 10^7 cfu. In the multichamber beads the further colonization with the formation of yeast microcolonies was observed. The amylolytic activity and ethanol production of free and immobilized cells *D. occidentalis* in starch media were comparable, but GC analysis of fermentation products showed different metabolic patterns.

Yeast 'nanobots'

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'Nanobots' or nanorobots are man-made devices that will be used in future to perform tasks in spaces that are invisible to the naked eye. It is predicted that they will have a diameter of about 0.5 to 3 microns and consist of parts ranging from 1 to 100 nm. We found that yeasts produce their own 'nanobots' in the form of sexual spores of similar dimensions. Here, yeast offspring is enclosed within capsules of different shapes (hat-, Saturn-, needle-, sickle-, walnut-, corkscrew- shaped) with nano-scale functional lubricated ornamentations such as fins, surface gears, compressible sheaths, corkscrew ridges, razor sharp brims and equatorial ridges. These capsules are in turn enclosed within yeast birth sacs (asci). These intriguing structures are not formed merely for identification purposes – yeasts have their own reasons for producing them. Our research suggests that many of these structures, coated with lubricating 3-hydroxy oxylipins, are necessary for effective release of ascospores from enclosed asci through water-propulsion (turgor pressure). Yeast 'nanobots' so far studied suggest gear-like (*Dipodascopsis uninucleata*), sliding (*Dipodascus*), drilling (*Eremothecium sinecaudum*), boomerang (*Eremothecium ashbyii*), piercing (*Eremothecium coryli*) and cutting (*Ascoidea corymbosa*) movements. What other types of 'nanobot' mechanics await to be discovered in yeasts? With the rescaling of these structures, they might be of value in the nano-, hydro- and aero- technologies. Already an effective fluid-driven pipe-cleaning device based on the structure of the 'nanobots' of the yeast *Dipodascopsis uninucleata* has been engineered. The application of 3-hydroxy oxylipins as lubricant in general mechanics should also be assessed and its characteristics compared to those on the market today.

Genomic screening for HMF-reducing enzyme(s) in *Saccharomyces cerevisiae*

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During the acid pre-treatment of lignocellulosic biomass for biofuel ethanol production, simple sugars are formed as well several types of yeast inhibitory compounds. Furans, like 5-hydroxymethylfurfural (HMF) and furfural, are the most important inhibitors present in the hydrolysate. They are known to inhibit yeast growth and viability and reduce ethanol productivity. Yeast strains are able to reduce HMF and furfural to less toxic compounds, however inhibitor rate conversion and cofactor uses are strain dependent. Using microarray analyses we have identified a first NADPH-dependent enzyme responsible for HMF conversion in *S. cerevisiae* (Petersson *et. al.* 2006). In the present work, we have constructed a genomic library using DNA from a tolerant *S. cerevisiae* strain and developed a screening method based on the increased HMF tolerance. *In vitro* and *in vivo* assays have been used to analyze HMF reduction capability of the selected strains. Physiological studies with such strains have been carried out under respire-fermentative conditions in order to analyze HMF uptake and strain tolerance.

Yeast as a model to study carbonyl reduction of anthracyclines

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Daunorubicin (DAU) and doxorubicin (DOX) are two anthracyclines commonly used in the treatment of cancer. The main metabolite of anthracyclines is formed through NADPH-dependent C-13 carbonyl reduction (Fig. 1). The resulting alcohol is significantly less potent in preventing tumor growth. Moreover it can induce both acute and chronic cardiotoxicity which can lead to heart failure long after the treatment has been stopped. Cardiotoxicity is dose-dependent and restricts the maximum allowed total dosage of the drug. The finding of an efficient inhibitor that could be used together with anthracyclines in the treatment of cancer would be of great interest for oncologists. In this work human carbonyl reductase 1 (HCBR1) was cloned into *Saccharomyces cerevisiae* from a human hepatoma cDNA library using PCR and its activity on anthracyclines was studied in crude cell extracts. DOX was not reduced by HCBR1 while DAU was. Inhibition of HCBR1 by various flavonoid compounds was investigated and inhibit kinetics results for inhibition will be presented.

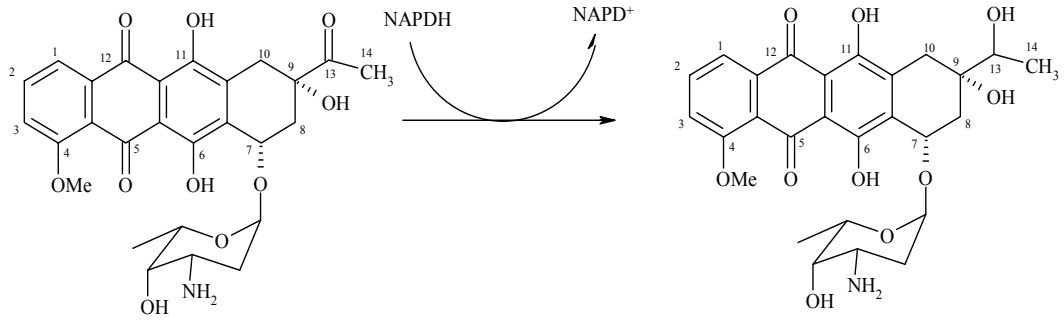

Figure 1. Carbonyl reduction of DAU.

The fungal path for D-galacturonic acid catabolism

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D-galacturonic acid is the major component of pectin and consequently an important carbon source for microorganisms living on decaying plant material or for biotechnological processes where cheap raw materials such as sugar beet pulp are used. A bacterial catabolic pathway has been described while a eukaryotic pathway has remained unknown. For *E. coli* a pathway was described consisting of five enzymes converting D-galacturonic acid to pyruvic acid and D-glyceraldehyde-3-phosphate. The enzymes of this pathway are uronate isomerase, NADH-utilizing D-tagaturonate reductase, altronate dehydratase, D-erythro-3-deoxy-D-hexulosonate kinase and D-erythro-3-deoxy-D-hexulosonate-6-phosphate aldolase.

We show that a fungal pathway exists that is distinctly different from any previously described pathway. In this pathway D-galacturonic acid is converted to pyruvate and glycerol. Intermediates are L-galactonate and L-threo-3-deoxy-hexulosonate. We have identified most of the enzymes of this pathway, cloned the corresponding genes, expressed them in a heterologus host and determined the kinetic properties of the enzymes. We will present potential biotechnological applications of this novel pathway.

Isolation and characterization of a new xylitol-producing yeast strain

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Xylitol production is one of the best way to valorize the hydrolyzates of lignocellulosic materials. Xylitol, a sugar alcohol derived from xylose, is equivalent to sucrose in sweetness. Moreover it is no cariogenic and is consumed by diabetics because it is metabolized by an insulin-independent pathway. Actually, the microbiological conversion of xylose into xylitol has been considered as a more economic alternative for xylitol production versus chemical process. In this perspective, from fermented redcurrants (fruit rich in xylose), we have isolated some yeast strains for their capacities to realize this conversion. Composition of synthetic media and cultural conditions were investigated to lead efficient xylose production and/or important biomass production. One strain has been selected and taxonomically characterized because of good biotechnological properties. It appears to be a promising yeast strain for xylitol production with a high yield.

Integrated studies on L-arabinose metabolism in yeasts

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Ethanol is considered one of the most promising alternative biofuels for transport and much attention is currently given to bioconversion processes from lignocellulosic materials. *Saccharomyces cerevisiae*, used in traditional fermentation industries, lacks the ability to utilise the predominant pentoses (D-xylose and L-arabinose) in the hemicellulose fraction. While efficient recombinant strains of *S. cerevisiae* expressing either the yeast or the bacterial xylose-metabolising pathway are already available, only recently the interest on the metabolic engineering of *S. cerevisiae* for arabinose fermentation based on the fungal and bacterial catabolic pathways was demonstrated. Notably, the characterisation of natural L-arabinose-utilising yeasts is still very poor.

Following an extensive screening of L-arabinose utilising yeasts, two strains (CFY 03 and CFY 12) showing rapid growth and high L-arabinose uptake rates were selected for further studies. The activity and specificity of all the enzymes involved in the L-arabinose catabolic pathway already described for filamentous fungi were determined and the results confirmed that this pathway is also operating in both yeasts. However, they show clearly distinct features. Despite the higher arabinose influx rate in CFY 12, CFY 03 displays significantly higher enzyme fluxes in the early steps of intracellular L-arabinose catabolism. In full agreement with this data, increased accumulation of arabitol and xylitol was observed with CFY 12 during experiments in batch cultures and using *in vivo* NMR with [2-^{13}C] L-arabinose. The cofactor imbalance, characteristic of the fungal arabinose degradation pathway, became obvious under oxygen-limited conditions and appears to determine the production of only trace amounts of ethanol from L-arabinose by yeasts.

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Towards the development of a process technology for making xylitol from glucose: optimization of D-arabitol production from glucose by a newly isolated *Zygosaccharomyces rouxii*

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Xylitol is a reduced-calorie sweetener with anticariogenic properties that does not need insulin for its metabolism by diabetics. Many yeasts have the capability to produce xylitol from xylose. The enzyme NADPH-dependent xylose reductase (EC 1.1.1.21) catalyzes the reduction of xylose to xylitol. We have studied the production of xylitol from xylose, mixed sugar substrates (xylose, arabinose, glucose), and corn fiber acid hydrolyzate by three yeasts (*Candida entomaea*, *Candida peltata,* and *Pichia guilliermondii*). However, these yeasts convert glucose to ethanol and L-arabinose to L-arabitol. There is a growing interest in the production of xylitol from a readily available, cheap substrate such as glucose. As a first step to produce xylitol from glucose via the D-arabitol route, we have isolated a yeast, *Zygosaccharomyces rouxii* NRRL B-27624, from honey bee hives. The yeast was able to utilize high concentrations of glucose and produce D-arabitol as the major metabolic product in a very good yield. In this presentation, the factors affecting the D-arabitol production by this yeast will be presented. The strategies for converting D-arabitol to xylitol by biotransformation and enzymatic means will be highlighted. The problems and prospects of developing a cost-effective fermentation process using yeast for production of xylitol from lignocellulosic hydrolyzates will be presented.

Development of industrial *Saccharomyces cerevisiae* strains co-utilising xylose and arabinose

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Saccharomyces cerevisiae is the preferred candidate for fuel ethanol production from lignocellulosic raw materials due to its ability to tolerate high ethanol concentrations and inhibitory compounds such as furans and acetic acid. Industrial yeast strains rather than laboratory strains have been shown to be more tolerant to such inhibitors, and are thus preferred for use in industrial applications. A trait required from a micro-organism used for ethanol production from lignocellulose is the ability to simultaneously utilize several sugars present in the lignocellulose hydrolysates. In this study, the xylose and the arabinose pathways together were both introduced in industrial *S. cerevisiae* strains. Strains able to grow on both pentose sugars were generated. The results on xylose and arabinose co-utilisation and fermentation will be presented.

Metabolic engineering of *Hansenula polymorpha* for high temperature alcoholic fermentation of xylose

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The development of a reliable and profitable technology for the conversion of lignocellulosic residues to fuel ethanol depends on the pretreatment and hydrolysis of biomass feedstocks to fermentable sugar streams. The most environmentally friendly hydrolysis process can be provided by cellulases and hemicellulases at high temperature around 50°C. The sugars produced from the hydrolysis step need to be removed from reaction mixture by simultaneous fermentation in the same vessel (so named SSF process). Since the optimal fermentation temperature of the currently available bacterial (*Escherichia coli, Zymomonas mobilis*) and yeast (*Pichia stipitis, Saccharomyces cerevisiae*) natural and recombinant strains that are capable of lignocellulose sugar fermentation is typically between 30° and 40°C, there is a need for new ethanolgens that can withstand higher temperatures for growth and ethanol production.

We have recently reported that the methylotrophic thermotolerant yeast *Hansenula polymorpha* (maximal growth temperature 49–50°C) can actively ferment the following sugars of lignocellulose: glucose, mannose, cellobiose and xylose to ethanol at 45–48°C. Fermentation is most active under limited cell aeration. One of the approaches we used for metabolic engineering of *H. polymorpha* to increase ethanol production from xylose involved the cloning of the bacterial xylose isomerase (*xylA*) gene. Successful expression of *xylA* gene in *H. polymorpha* has been obtained in knock out mutants defective in xylose reductase and xylitol dehydrogenase. Corresponding transformants did not differ from the wild-type strain regarding ethanol accumulation; however, secondary mutants producing larger colonies in xylose medium have been obtained which produced significantly elevated amounts of ethanol. Another approach we used involved site-specific mutagenesis of *XYL1* gene (yeast gene responsible for D-xylose to xylitol conversion) to increase the affinity of xylose reductase to NADH and decreasing that to NADPH, resulted in increase of ethanol production in xylose medium. A new auxonographic method for isolation of the mutants accumulating elevated amounts of ethanol in xylose medium has been developed. Some of the isolated mutants are defective in different steps of ethanol catabolism, others display impairment in regulation of fermentation process. Other strategies for further improvement of the fermentation characteristics will be discussed.

Successful expression of bacterial *xylA* gene encoding xylose isomerase from *Escherichia coli* in the methylotrophic yeast *Hansenula polymorpha*

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Fuel ethanol production from the renewable raw material, such as plant biomass or lignocellulose, has a great economic and ecological significance. Yeast, which are able to ferment xylose, a major component of lignocellulose biomass, can carry this out using NAD(P)H dependent xylose reductase and NAD dependent xylitol dehydrogenase. This results in redox imbalance causing a low efficiency of the xylose fermentation and accumulation of large amounts of xylitol instead of ethanol. One of the ways to bypass the redox imbalance problem for efficient xylose fermentation is to express the bacterial xylose isomerase gene in yeast. The thermotolerant methylotrophic yeast, *Hansenula polymorpha,* is able to ferment xylose to ethanol. We report the successful demonstration of the expression of bacterial *xylA* gene coding for xylose isomerase from *E. coli* in *H. polymorpha*. The expression was achieved by integration of the *xylA* gene driven by the promoter of the *H. polymorpha* glyceraldehyde-3-phosphate dehydrogenase gene (*HpGAP*) into the *H. polymorpha* genome. Expression of the bacterial xylose isomerase gene restored the ability of the *H. polymorpha Δxyl1, Δxyl1 Δxyl2-A* or *Δxyl1 Δxyl2-A Δxyl2-B* strains to grow in a medium with xylose as a sole carbon source. The mutants with a deletion of the *XYL2-A* gene displayed near 70% of xylitol dehydrogenase activity of that in the wild-type strain. The *H. polymorpha Δxyl1*(*xylA*) and *Δxyl1 Δxyl2-A*(*xylA*) transformants displayed xylose isomerase activities, which were near 25% of that of the bacterial host strains. The transformants did not differ from the yeast wild-type strain with respect to ethanol production in xylose medium. We suggest that *H. polymorpha* second xylitol dehydrogenase gene (*XYL2-B*) is responsible for conversion of part of accumulated in xylose isomerase reaction xylulose back to xylitol, which inhibits the xylose isomerase activity. In the xylose-containing medium spontaneous colonies selected for increased ability to grow were analysed. These mutants have shown near three fold higher ethanol production in xylose medium of that in the wild-type strain. The mutants with a deletion of the *XYL2B* gene displayed near 35–50% of xylitol dehydrogenase activity of that in the *Δxyl1 Δxyl2-A* strain. The strains *Δxyl1 Δxyl2-A Δxyl2-B*(*xylA*) displayed xylose isomerase activities, which were near 80% of that of the bacterial host strains. The ethanol production of these mutants was higher as compare with wild-type strain.

Systems biology of yeast: Genome-wide analysis of pentose metabolism in *Saccharomyces cerevisiae*

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We are interested in broadening the substrate utilisation range of the yeast *Saccharomyces cerevisiae* and in connection to this to study nutritional responses and signalling at a global level. *S. cerevisiae* does not naturally utilise pentose sugars unlike most other fungi, and recombinant *S. cerevisiae* strains have been constructed that contain the xylose utilisation pathway from other yeasts. The xylose reductase enzyme (XR) prefers NADPH as a cofactor while the next step in the pathway xylitol dehydrogenase (XDH), NAD^+. This is believed to create a redox cofactor imbalance in the cells and limit xylose fermentation. Other suggested rate-limiting steps in xylose utilisation and the use of xylose as a fermentative carbon source include xylose uptake and limitations in the pentose phosphate pathway (PPP) reactions.

In order to understand redox and nutrient regulation in general, and the physiology of xylose utilising *S. cerevisiae*, we carried out chemostat cultures on xylose as a carbon source, and compared those with glucose cultures. Transcriptional profiling and proteomics were carried out. The results are in good agreement. In addition to the expected responses in cellular redox metabolism and PPP, also new responses towards xylose as a carbon source were discovered such as the up-regulation of pathways for alternative carbon source utilisation and responses for nutritional control and starvation. The physiology of the recombinant yeast appears to be neither fully repressed (fermentative) nor derepressed (gluconeogenic).

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Efficient production of L-lactic acid from xylose by metabolically engineered yeast *Pichia stipitis*

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Pichia stipitis, a yeast which ferments naturally xylose, was genetically engineered for L-(+)-lactate production. A *P. stipitis* strain expressing the L-lactate dehydrogenase (LDH) from *Lactobacillus helveticus* under the control of the *P. stipitis* fermentative *ADH1* promoter was constructed. Either xylose or glucose was used as the carbon source for lactate production under oxygen restricted conditions. Remarkably, the constructed *P. stipitis* strain produced a higher lactate concentration and yield on xylose than on glucose. Lactate accumulated as the main product on xylose-containing medium: 58 g/l lactate was produced from 100 g/l xylose. Relatively efficient lactate production was also observed on glucose medium, 41 g/l lactate was produced at a yield of 0.44 g/g glucose. Lactate was produced at the expense of ethanol production which was decreased to approximately 20% of the wild type levels on xylose-containing medium and to 75% on glucose-containing medium. Thus, LDH competed efficiently with the ethanol pathway for pyruvate, even though the pathway from pyruvate to ethanol was intact.

Online intracellular ph measurements upon sorbic acid stress in yeast

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The weak organic sorbic acid (pKa=4.76) is the most widespread preservative used in the food industry. Yeast and other fungi are to a certain extent able to adapt to this acid and resume growth in the presence of the highest concentrations allowed in foods. This can result in substantial economic loss. At low pH a substantial amount of the acid is uncharged, and can diffuse freely over the plasmamembrane. A mechanistic model for weak acid stress has been around for quite some time, and is based on 4 possible mechanisms for growth inhibition. However, a good quantitative model describing the growth inhibition and adaptation to sorbic acid stress based on experimental data remains to be produced. By mapping the different possible components of weak acid stress we try to identify putative targets and defense mechanisms in yeast. One of the main components may be intracellular acidification. To understand sorbic acid stress kinetics we need to measure intracellular acidification *in vivo*, on a time-scale of both seconds and hours, and be able to distinguish between various organelles. Therefore, we use the pH sensitive GFP pHluorin to determine intracellular pH (Miesenböck *et al*. 1998). Initial experiments showed a stable cytosolic pH of 7.3 in yeast cells grown on glucose at pH 5 in a defined mineral medium in logarithmic growth phase. A stable mitochondrial pH of 7.6 was measured under these conditions. Lowering the pH of the medium to 3.0 with a strong acid did not have an effect on intracellular pH, neither did an adjustment to neutral pH. In contrast, addition of sorbic acid resulted in an immediate drop in cytosolic pH. Importantly, mitochondrial pH under these conditions also dropped to the same level resulting in a possible dissipation of proton motive force. After 2 hours intracellular pH started to recover although not fully to pre-stress levels. These findings correlate nicely with growth measurements where cells undergo a lag phase and resume growth with a lower growth rate. These measurements give new insights in weak acid stress kinetics and provide valuable new data for modeling purposes.

Engineering redox metabolism in *Saccharomyces cerevisiae* by expression of a H_2O-forming NADH oxidase

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To analyse how *Saccharomyces cerevisiae* metabolism during growth on glucose might be altered when intracellular NADH pool is decreased, we recently expressed *noxE* encoding a H_2O-NADH oxidase from *Lactococcus lactis* in the *S. cerevisiae* V5 strain. Another goal of this study was to evaluate a strategy based on cofactor engineering to reduce the ethanol content in wine.

During batch fermentations under controlled microaeration conditions, expression of the oxidase, which catalyses the oxidation of NADH by reducing molecular O_2 to H_2O drastically reduces the intracellular NADH concentration and substantially alters the distribution of metabolic fluxes. The yields in ethanol, glycerol and hydroxyglutarate were significantly decreased, whereas the formation of more oxidized metabolites, acetaldehyde, acetate and acetoin was favored. As the result of ADH limitation, a high level of acetaldehyde accumulates early in the process, impairing growth and fermentation performance[1]. To further understand these effects, we have carried out a comprehensive analysis of the impact of oxygen on the metabolic network of the NADH oxidase-expressing strain. While reducing the oxygen transfer rate led to a gradual recovery of the growth and fermentation performance, the impact on ethanol yield was negligible. In contrast, supplying oxygen only during the stationary phase resulted in a 7% reduction in the ethanol yield, but without affecting growth and fermentation[2]. Importantly, our data also point to a significant role for NAD^+ reoxidation in controlling the glycolytic flux.

In addition to providing new insights into redox metabolism, this strategy offers perspectives for industrial applications. Strains expressing a NADH oxidase are a potentially valuable system for reducing the ethanol content in wine, as well as for optimal production of fine chemicals, which requires effective regeneration of cofactors.

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NADP dependent glycerol dehydrogenases in the mould *Hypocrea jecorina* and their application in cofactor engineering

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Fungal microorganisms have the enzymes for a glycerol cycle consisting of the following enzymes: glycerol dehydrogenase (NADP+), dihydroxyacetone kinase, glycerol-3-phosphate dehydrogenase (NAD+) glycerol-3-phosphate phosphatase. In each cycle NADPH and NAD is formed from NADP and NADH at the expense of ATP. However an active glycerol cycle has never been reported. If active, such a cycle could be used in cofactor engineering. An application could be pentose fermentation with the yeast *S. cerevisiae* where the NADPH/NADP imbalance is a rate limiting factor. Our approach was to express an NADP dependent glycerol dehydrogenase and the endogenous DAK1 (dihydroxy acetone kinase) from a constitutive promoter as a strategy to introduce this glycerol cycle in yeast.

NADP glycerol dehydrogenases can convert glycerol to glyceraldehyde or dihydroxy acetone. So far it was not possible to predict whether a glycerol dehydrogenase was glyceraldehyde or dihydroxy acetone forming. The genes gld1 and *gld2* from mould *Hypocrea jecorina* (*Trichoderma reesei*) coding for enzymes with high similarity to the NADP-dependent glycerol dehydrogenases were cloned and expressed in a heterologous host. The encoded proteins were purified and their kinetic properties characterized. The GLD2 characteristics are similar to the previously described NADP-dependent glycerol-2-dehydrogenases (EC 1.1.1.156) purified from different mould species. It is a reversible enzyme active with dihydroxyacetone or glycerol as substrates. The GLD1 (EC 1.1.1.72) catalyses the conversion of D-glyceraldehyde and L-glyceraldehyde to glycerol, however there is tiny activity in reverse reaction.

The GLD2 was chosen for overexpression together with DAK1 to facilitate the glycerol cycle in *S. cerevisiae*. Preliminary studies on xylose fermenting *S. cerevisiae* will be presented.

Quantitative analysis of signalling through the two HOG pathway branches

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The yeast *Saccharomyces cerevisiae* senses osmotic changes through the HOG MAP kinase network. The Hog1 protein kinase then mediates transcriptional as well as post-transcriptional responses. The kinase is activated by Pbs2, which in turn is controlling by two distinct sensing branches, the Sln1 and the Sho1 branch. The underlying mechanisms of osmosensing are not well understood but it appears that the two branches interpret osmotic changes in different ways. We have previously presented a comprehensive data-supported mathematical model of the HOG signalling pathway from sensing to response; however, this analysis was restricted to the Sln1 branch.

In this work we set out to compare in detail the behaviour of the two branches separatly in order to feed a mathematical model to be generated by the group of Edda Klipp. Such a model will then be used to overcome an important limitation of experimentation: the output via the two separate branches can only be measured in mutants lacking one branch, which may give an imperfect impression of their behaviour and contribution in wild type cells.

We have studied pathway activation and deactivation of the two branches separatly, measuring in time course Hog1 phosphorylation, Hog1-dependent gene expression and glycerol accumulation. Both branches display similar activation/deactivation profiles following osmotic treatment. In addition, both branches can readily be re-activated by a second osmotic treatment, indicating that feedback regulation works such to ensure signalling competence. Interestingly, however, in mutants unable to accumulate glycerol only the Sln1 branch shows hyperactivation and prolonged activation, while the Sho1 branch activity rather seem to be downregulation. This hints to different feedback control mechanisms.

In addition, we are setting out to study response mechanisms to osmotic treatments at single cell level. We will present first results of a study employing microfluidic systems and optical tweezers.

A comparative study of the yeast HOG pathway

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Comparative genomics allows the comparison of different proteins that execute presumably identical functions in different organisms. In contrast to paralogues, orthologues per definition perform the same function and interact with the same partners. Consequently, they should display conservation not only in primary sequence but also in domain organisation and protein size. The sequencing of numerous fungal genomes has offered the possibility to compare genomes over a degree of evolutionary divergence. Additionally, the large number of sequenced genomes provides for a statistical analysis of orthologue size in addition to primary sequence, providing a new tool for interspecies sequence comparison. Here, we report a comparative analysis of the yeast HOG pathway, defining differences in the signalling pathway architecture between species, such as absent components or components with altered domain structure. Such comparative analysis provides a powerful complement to functional protein analysis, such as discovery of novel, and evaluation of characterised, functional domains, as well as evaluation of potential sites for posttranslational modification. The fungal genome sequences provide a unique resource that should facilitate the functional analysis of any fungal proteins.

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Integrated analysis of the response of yeast cells to osmotic stress – contribution of selected mechanisms to signal cessation via HOG pathway

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Osmoregulation, the active control of cellular water homeostasis by cells and organisms, is a fundamental biological process crucial for life. The yeast *Saccharomyces cerevisiae* serves as an excellent model system to study the molecular biology and physiology of osmoadaptation. Substantial amount of information has already been collected on *S. cerevisiae* osmotic-shock-induced signal transduction, gene expression control and osmolyte accumulation. Two distinct yeast transmembrane proteins Sln1 and Sho1 appear to be independent osmosensor devices, controlling two independent branches within the HOG (High Osmolarity Glycerol response) pathway. The HOG pathway shares similarity with MAPK pathways conserved throughout eukaryotes. Upon shift to high osmolarity, yeast cells rapidly stimulate the HOG MAPK system, which orchestrates part of the transcriptional response. Since prolonged activation of HOG pathway is detrimental, the decisive event in downregulation of the osmosensing yeast HOG pathway is signal termination.

We have recently published a mathematical model of the response of yeast to osmotic shock (Klipp *et al.* 2005) which integrates results of simulations and experimental data on the Sln1 branch. The model provides evidence that downregulation of the HOG pathway, and thus successful osmoadaptation is mediated by *glycerol accumulation* which leads to cell reswelling and increase in turgor.

The following mechanisms contribute to glycerol accumulation in yeast cells immediately after an osmotic shock: (i) rapid closure of Fps1 (an aquaglyceroporin that mediates osmolarity-regulated passive glycerol flux); (ii) increased Hog1-dependent expression of *GPD1* and *GPP2* encoding dehydrogenases/phosphatases involved in glycerol production; (iii) activation of 6-phosphofructo-2-kinase increasing glycerol production via enhanced glycolytic activity. The aim of the work was to analyse the three presumably main control mechanisms in glycerol accumulation, updata data on the previously published model and hence work towards a complete, integrated description of the HOG system.

Klipp, E. et al. (2005) Integrative model of the response of yeast to osmotic shock. Nature Biotech. 23: 975–982.

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Glycerol transport in halophilic *Debaryomyces hansenii*

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The physiological characterization of glycerol uptake putatively coupled to a gradient of Na^+ in *D. hansenii* was previously described (Lucas *et al*. 1990). In yeasts, defense responses to salt stress are based on osmotic adjustment by osmolyte synthesis and cation transport systems for sodium exclusion (Blomberg and Adler, 1992). In *Saccharomyces cerevisiae*, physiological studies have previously shown the presence of a glycerol active uptake system driven by proton motive force (Bjúrn *et al*. 1992), only operative when glycerol is the carbon source; Ferreira *et al*. 2005, reported that the glycerol proton symporter in *S. cerevisiae* is encoded by *STL1*. It is also interesting that *D. hansenii* has the natural capacity to adapt to high concentrations of salt, Na^+ being accumulated without producing any apparent toxicity. On the contrary, cells show some better functional characteristics in the presence of NaCl (Prista *et al*. 1997, González-Hernández *et al*. 2004). Considering the important role of glycerol in osmoregulation described for *D. hansenii* (Adler and Gustafsson, 1980; González-Hernández *et al*. 2005), our objective is to characterize the glycerol transport from both molecular and physiological point of view. We investigated the existence of glycerol transport genes (*GUP1* and *STL1*) in *D. hansenii* using online database of Génolevures consortium. We designed primers in order to amplify *D. hansenii GUP1* and *STL1* ORF and their regulatory regions. *D. hansenii* genomic DNA was isolated after a previous treatment with liticase. Standard molecular biology methods for plasmid isolation and DNA analysis were employed. Using this approach, we amplified two fragments of 2.8 and 2.6 Kb from *D. hansenii* genomic DNA. The fragments were cloned, characterized at molecular level, and used to transform *gup1*, *gup2*, and *stl1* deletion *S. cerevisiae* strains in order to perform physiological characterization of the resulting phenotype. The ability of yeast strains to grow in the presence of high NaCl, KCl, glycerol and sorbitol concentrations on solid YNB-ura media were evaluated. Kinetic characterization of glycerol transport is being carried out, and northern blot techniques will be performed, in order to understand the role of the *GUP1* and *STL1* genes of *D. hansenii*.

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Polymorphous transitions in “black yeasts” phaeococcomyces chersonesos: hyperosmolarity vs starvation

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We study cell morphology in *Phaeococcomyces chersonesos* cultures grown on variable stressful media, including fluctuations of osmolarity and nutrient availability. Visual analysis reveals that osmotic stress effects notably dominate on combined media, leading to formation of similar phenotypic picture irrespective of concomitant nitrogen\glucose levels. In a number of cases system effects may be observed, i.e. resulting effect of stressors acting in concert can not be reduced to summation of their particular effects, probably due to signaling “cross-talks”. Morphometric data on several parameters (average number of cells in chains, plan area value distribution, etc.) was obtained and statistically processed via VideoTesT-Morphology 4.0 software. Using tree-structured clusterization algorithm (Statistica 6.0) set distances between properties of compared cell cultures were calculated to estimate different stress-factors influence (each taken separately and in all possible combinations) on cell morphology. So, different stressful media were classified according to morphological proximity of corresponding cell cultures. Thus it came out that osmotic stress is really the most significant in respect of morphogenesis, followed by nitrogen deficiency, and then by carbohydrate starvation. In terms of systems theory osmostress influence can be called “power-switching”, whereas nutrient availability fluctuations are parametric, i.e. more delicate, possessing quantitative characteristics.

We used these data to develop a prognostic mathematical model (*FuSTRE*) describing *P. chersonesos* growth under different nitrogen, sugar and salt content. *FuSTRE* implies stress-induced activation of two signalling blocks with presumable antagonistic influence on cell morphology. It is supposed that these blocks include components of adenylate cyclase cascade and MAP-kinase HOG-like module, therefore main factors, crucial for cell morphology determination, are marked as MAPK и PKA. All processes are described in single first order differential equations. There are as yet some admissions, namely: a) yeast\mycelium breeding rate scale concerning only with carbohydrates content b) MAPK dynamics depending only on salt (NaCl) content c) yeast\mycelium breeding ratio depending on MAPK- as far as on PKA-activity, with multiplicative action of given MAPK\PKA-dependent blocks. First version of the simulator program based on given algorithm was created; approbation data mainly agree with experimental.

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Multiple functions of Na^+/H^+ antiporters in yeast

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In all organisms, Na^+/H^+ antiporters serve to maintain low intracellular concentration of toxic sodium cations (bacteria, yeast, plants) or to eliminate surplus protons from cytosol (mammals). *Saccharomyces cerevisiae* possesses three types of Na^+/H^+ antiporters, one in the plasma-membrane (Nha1p) and two in the membranes of intracellular organelles (Kha1p, Nhx1p). All of them probably transport not only toxic alkali metal cations but also potassium. *In silico* search revealed the conservation of homologous genes in other yeast species, and the heterologous expression in *S. cerevisiae* cells confirmed their roles in transport of alkali metal cations and protons. The intracellular antiporters are important for sequestration of surplus alkali metal cations in the vacuole (Nhx1p) and for survival of cells at high external pH (Kha1p). Their activity regulates potassium content and pH in the organelles of endosomal/prevacuolar compartments (Nhx1p) and Golgi apparatus (Kha1p). This way, the intracellular antiporters are involved in the regulation of cell protein trafficking. The plasma-membrane Nha1p is the main system eliminating surplus cations from cells at acidic external pH values. Besides this detoxification role, its transport activity contributes to maintenance of intracellular potassium and pH homeostasis, and participates in regulation of intracellular pH and cell volume, in formation of plasma membrane potential and in the response of cells to a sudden osmotic shock. Our results show that transport systems with potassium/proton antiport mechanism play an important role in yeast physiology and are involved in many cell functions.

Proteomic mapping of *Debaryomyces hansenii* used for evaluation of changes in protein expression upon NaCl stress

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The highly osmotolerant yeast *Debaryomyces hansenii* has been isolated from different environments such as sea water, brines, and salted food products including different types of cheeses and meat products, where it contributes to development of the bacterial flora, aroma and colour stabilisation and inhibition of undesirable contaminants (moulds and pathogens such as *Listeria monocytogenes*). The physiological properties of *D. hansenii* have been investigated to some extent, however, the genetic and proteomic knowledge are insufficient. Recently, the genome of *D. hansenii* has been fully sequenced, and sequences are available at http://cbi.labri.fr/Genolevures/elt/DEHA. In this study, the proteome of *D. hansenii* has been investigated by two dimensional polyacrylamide gel electrophoresis (2-D PAGE), and protein spots have been identified by matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) in order to make the first proteomic map for *D. hansenii*. For investigation of the influence of NaCl on the proteome, proteins were labelled with L-[^{35}S]methionine prior to protein extraction and separation. Several proteins were identified being either induced or repressed upon exposure to NaCl. Induced proteins were enzymes involved in glycerol synthesis/dissimilation and upper part of glycolysis, whereas repressed proteins were enzymes involved in lower part of glycolysis, route towards Krebs cycle and synthesis of amino acids. Furthermore, both inductions and repressions among heat shock proteins were observed upon NaCl exposure. The identification of the proteome of *D. hansenii* will add to the understanding of how this NaCl tolerant yeast species deals with NaCl stress.

Transcriptome analysis of Met4p-mediated sulfur regularoty circuit in the methylotrophic yeast *Hansenula polymorpha*

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Sulfur plays important roles in cellular processes, not only as a main component of biomass but also as an antioxidant when exposed to cadmium. The global transcriptional activator, Met4p, plays crucial roles in both sulfur assimilation and sulfur-sparing response to cadmium in *Saccharomyces cerevisiae*. To obtain comprehensive overview on the sulfur regulatory networks in the methylotrophic yeast *Hansenula polymorpha*, we constructed the *H. polymorpha MET4* gene deletion strain (Δ*hpmet4*) and performed transcriptome analysis under sulfur limitation and cadmium exposure conditions. Compared to the wild type, the *Δhpmet4* strain showed more dramatic changes of gene expression under these stress conditions, indicating that the function of *HpMET4* is essential for the cell homeostasis in sulfur depletion condition and in cadmium detoxification. Those genes, whose expressions were highly up-regulated in the wild-type strain under both conditions but not so much changed or rather decreased in the *Δhpmet4* strain, were defined as the putative *HpMET4*-regulons. The promoter analysis of these genes, especially a set of *MET* family genes of *H. polymorpha,* revealed the presence of common motif for the Cbf1 binding site (TCACGTG), which strongly indicates that the expression of these genes are regulated by HpMet4p in association with Cbf1p in *H. polymorpha*.

The Cdk-inhibitor Sic1 is a major molecular target of TOR pathway that mediates rapamycin-induced G1-arrest in *Saccharomyces cerevisiae*

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A tight coordination of cell growth and division is of outmost physiological relevance and it is responsible for cell size homeostasis (Rupes, 2002). In the budding yeast *Saccharomyces cerevisiae*, cells have to reach a critical cell size at Start which is modulated by carbon source, to enter into S phase (Wells, 2002). Recently we identified the molecular basis of the cell sizer mechanism in budding yeast. The cell sizer machinery has been shown to require the overcoming of two sequential thresholds, involving Cln3 and Far1, and Clb5,6 and Sic1, respectively (Alberghina *et al.,* 2004; Rossi *et al*., 2005). Studies in budding yeast have provided important insights into the physiological role and regulation of the TOR (Target of Rapamycin) pathway and its connection to cell size and growth control, revealing that its activation signals favourable nutrient conditions and promotes cell growth and proliferation (Schmelzle *et al.*, 2004). Here we identified the pathway in the regulatory network that mediates rapamycin-induced G1-arrest, studying quantitatively the pattern of expression of the cyclins Cln3, Cln1, Cln2 and Clb5, of the Cdk Cdc28 and of the Cdk-inhibitor protein Sic1 in a time course after the treatment with rapamycin. We show the involvement of both nutrient dependent thresholds in the regulation of cell cycle progression by TOR pathway. It is the up-regulation of *SIC1* at transcriptional and post-translational level and its nuclear accumulation, together with the downregulation of G1 cyclin expression, that results in the G1 arrest caused by TOR inactivation. These findings will be incorporated in our mathematical model of the G1 to S transition. Their modeling would improve the accuracy of this model (discussed in an accompanying poster), checking its reliability by considering effects of this specific signaling pathway on cell cycle progression.

Do different yeast stress responses share common physiological adaptations?

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Different types of changing environmental conditions trigger specific shifts in the gene expression profile of yeast. Ultimately, changes at the transcriptional level reflect the efforts of the cell to build the adequate adaptive response. Part of the yeast's adaptive response can be explained by the need to reach a new physiological state, with new values for flux and metabolite concentrations. Traditionally, this part of the adaptive response has been well studied, thus providing a significant amount of data. This date is invaluable for the study of yeast stress responses from a systemic point of view. Theoretical approaches offer the chance of using this data to explore evolutionary constraints to the changes in gene expression profiles.

In a previous work (BMC Bioinformatics 2006, 7:184) we evaluated the quantitative performance of gene expression profiles in yeast heat shock response by using eight functional criteria (flux changes, buffering of intermediate metabolite concentrations, protein cost as changes in gene expression occur, etc.). Altogether, those criteria explain the experimentally observed gene expression profiles in the heat shock adaptive response of yeast. We now apply these criteria to study gene expression profiles in yeast for other type of adaptive responses. First, we collected sets of gene expression profiles that are published in the literature for various types of stress, such as osmotic stress, acidic stress, oxidative stress, cold shock, etc. Then, we applied the same functionality criteria as before to study the gene expression profiles for the different types of stress. There is only partial overlap in quantitative criteria that are met by gene expression profiles for each type of stress to those for that met for heat shock,. This gives a measure of the "universality" and "particularity" of each criterion as a functional constraint for the responses to different types of stress, while simultaneously providing a measure of the distance between the response to the different types of stress. A clustering analysis of the pattern of distances reveals patterns and similarities that can shed light on phenomena such as cross-tolerance.

Rsp5p ubiquitin ligase is involved in lipid biosynthesis in yeast

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Rsp5 ubiquitin ligase is involved in many cellular processes including the regulation of unsaturated fatty acids biosynthesis through its role in the transcriptional activation of the essential Δ-9 fatty acid desaturase gene (*OLE1*). When Rsp5p is not functional, growth relies on the supplementation of the medium with oleic acid. We found that transcription of *PIS1* (encoding phosphatydylinositol synthase) and *INO1* genes (encoding inositol synthase) was increased about 2 fold in *rsp5* mutant compared to the wild type cells as shown by transcriptional reporter fusions: P_{PIS1}-*lacZ* and P_{INO1}-*lacZ*.

In order to identify other genes involved in the lipid biosynthesis and responsive to Rsp5p depletion, we performed genome-wide microarray analysis of gene expression in *rsp5* mutant strain at restrictive (37ºC), semirestrictive (33ºC) and nonrestrictive (22ºC) temperature. Interestingly, we did not detect any significant changes in gene expression at nonrestrictive temperature what suggest that mutated protein was rather functional in this condition. At 33°C and 37°C expression of 85 and 115 genes, respectively, was significantly changed when compared to the wild type cells grown in the same temperatures. We found a group of 11 genes whose expression was changed in both conditions. One of them, *LCB5* is involved in lipid metabolism and encodes minor sphingoid long-chain base kinase. The other genes from this group were involved in meiosis, amino acid metabolism and rRNA processing. Among the genes that were responsive exclusively at 33°C we identified 5 involved in lipid metabolism: *FAB1* encoding 1-phosphatidylinositol-3-phosphate 5-kinase, *VPS38* encoding subunit of a phosphatidylinositol 3-kinase complex, *ATG3* encoding protein involved in autophagy, which plays a role in formation of Atg8p-phosphatidylethanolamine conjugates, *SKN1* involved in sphingolipid biosynthesis, *IZH4* encoding membrane protein, which expression is induced by fatty acids and altered zinc levels, with a possible role in sterol metabolism. Among the genes that were responsive exclusively at 37°C we identified 2 other genes related to lipid metabolism, *SCS22* and *PDR17*, both encoding proteins involved in lipid traffic.

All together, these previous data support the notion that Rsp5 ubiquitin ligase does play a role in lipid metabolism, and that the transcriptional response to Rsp5p depletion may represent a compensatory mechanism allowing the cells to compensate for lipidic perturbations.

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International Specialised Symposium on Yeasts ISSY25
Systems Biology of Yeasts – from Models to Applications

Abstract

The 25th International Specialised Symposium on Yeasts, ISSY25, dedicated to "**Yeast systems biology – from models to applications**" is a symposium in a series organised by the members of the International Commission on Yeasts (ICY), an IUMS organisation. The ISSY25 Symposium has 240 participants from 29 different countries worldwide.

Systems biology is expected to have a major impact on the future of biological research. *Saccharomyces cerevisiae* is one of the best eukaryotic organisms for systems biology studies. System-wide analyses can be performed rapidly and accurately, followed by data integration and model construction. In addition, the number of yeast genome sequences is increasing rapidly. This provides an excellent opportunity for gaining insight into genomic diversity, and how this contributes to the phenotypic differences and industrially relevant properties of various yeast species. The symposium discusses the achievements, challenges and future prospects of yeast systems biology, and how the research community should best use its efforts to create understanding of eukaryotic microbial life.

The main financial supporters of the meeting are YSBN Yeast Systems Biology Network EU-Project (LSHG-CT-2005-018942), FEMS, The Academy of Finland, and Lipid Biotech (University of the Free State SA).

Keywords
systems biology, yeast, genomics, *Saccharomyces cerevisiae*, modelling, proteomics, metabolomics, microbial physiology, metabolic engineering

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