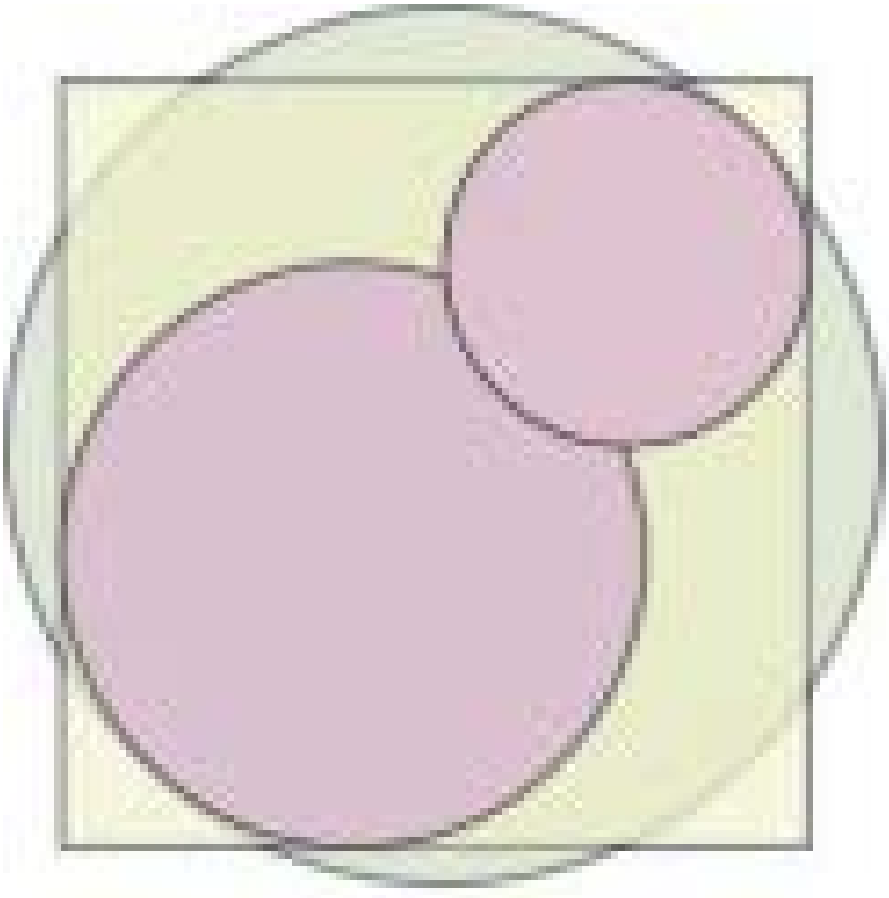
ICSB 2005

The Yeast Systems Biology Network Workshop



Sunday 23rd October
TMEC Amphitheatre 9:00-17:00

Program

Time	Name	Title
9:00-9:40	Bernhard O. Palsson	The construction of an integrated metabolic and transcriptional regulatory network
9:40-10:00	Joseph Lehar	Probing biological networks using chemical combinations
10:20-10:40	Suzanne Komili	Translational regulation defined by nuclear determinants and duplicated ribosomal proteins
Coffee		
11:00-11:40	Fritz Roth	Network analysis of synthetic lethal genetic interactions
11:40-12:20	Alain Nicolas	SGA screens identify genetic network interaction among replication (rad27), double strand break repair (rad52) and nuclear pore (nup133) deficiencies in yeast
Lunch & Posters		The Posters can be viewed in the room just outside the TMEC Amphitheatre
14:00-14:40	Alejandro Colman-Lerner	Regulated cell to cell variation in a cell fate decision system
14:40-15:00	Matthew J. Bauer	Homeostatic adjustment and metabolic re-modeling in a glucose limited yeast culture
15:00-15:20	Risto Renkonen	Dynamics in induced repression of phosphomannose isomerase PMI40 gene of *S.cervisiae*
Coffee		
15:40-16:20	Edda Klipp & Lilia Alberghina	Integrative analysis of the cell sizer network controlling entrance into S phase: role of the nucleo-cytoplasmic localization of Sic1
16:20-16:40	Michael Samoilov	Enhanced biomolecular network reconstruction via use of heterogeneous data
16:40-17:00	Gunnar Cedersund	In vivo identification of yeast glycolysis

Abstracts for talks

The construction of an integrated metabolic and transcriptional regulatory network

Bernhard Palsson

Univeristy of California, S.Diego, USA

Probing Biological Networks using Chemical Combinations

J. Lehár[1,2], G. Zimmermann[1], A. Krueger[2], L. Giusti[1], B. Stockwell[3], and C. Keith[1]

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Chemical combinations can provide rich constraints on biological systems[1]. Pairwise genetic deletions in yeast have already shown that combinations reveal functional associations between genes[2], and chemical screens have been able to distinguish between yeast mutant strains[3]. Our screens for novel combination therapies[4] produce response surfaces whose shapes differ markedly between mechanisms (Fig. 1). This information could be especially useful for systems biology because: (1) phenotypic responses to variable doses yield more detailed combination effects than those from basically digital genetic screens; (2) chemical probes target cellular components at the protein level, providing different and more immediate constraints on cellular function; and (3) chemical combination approaches can be applied to disease-relevant systems, like human signaling, that are less amenable to genetic deletions. We investigated the utility of chemical combinations through numerical simulations of metabolic pathways in conjunction with a small yeast screen of combined antifungal treatments targeting the sterol and other pathways. The simulations produced distinct response surface shapes for differing target configurations (Fig. 2), and the observed yeast proliferation responses are fully consistent with those predicted for the known topology and regulation of the sterol pathway in yeast. This work confirms that chemical combinations provide sensitive new constraints on the existence *and nature* of functional connections between targets, which can be used for systems biology and chemical genetics applications.

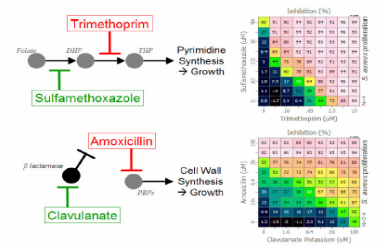

Fig. 1: Mechanism and dose responses for the antibiotic combination drugs Bactrim® (above) and Augmentin® (below).

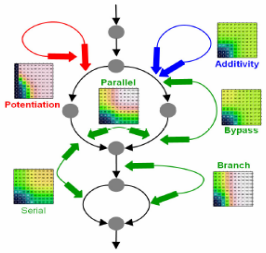

Fig. 2: Simulated dose response surfaces produced by applying paired inhibitors (arrows) to a branched metabolic pathway.

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Translational Regulation Defined by Nuclear Determinants and Duplicated Ribosomal Proteins

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Regulated translation is essential for establishing cellular polarity, which is required fordevelopment, cellular differentiation, and neuronal plasticity. *S. cerevisiae* is a modelsystem for the study of such processes since it regulates mating type in the daughter cell through polarized localization of certain mRNAs and their corresponding proteins. By studying a nuclear factor essential for proper mRNA localization in the cytoplasm, we demonstrate that several translationally regulated mRNAs need to be marked for translational repression prior to nuclear export, and that the regulated translation of these transcripts requires a specific subset of duplicated ribosomal protein genes. Genomic
mRNA-immunoprecipitations further suggest that this mechanism extends to other transcripts, including those of cell-cycle regulatory factors. Our data yield a model in which certain transcripts are bound within the nucleus by a translation factor that subsequently recruits a non-canonical cytoplasmic ribosome composed of a distinct subset of duplicated ribosomal proteins. These non-canonical ribosomes enable regulated translation of target mRNAs and can affect both the spatial and temporal properties of their translation. Thus, we report the first functional explanation for the existence of multiple copies of ribosomal protein genes. Additionally, the mechanism we describe may generalize to higher eukaryotes and should have a significant impact on the modeling of gene expression.

Network analysis of synthetic lethal genetic interactions

Frederick "Fritz" Roth

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Two genes have a synthetic lethal interaction if mutations in each gene separately are tolerable, but simultaneous mutation in both genes causes cell death. Such interactions provide robustness of an organism to mutation. We examined synthetic sick or lethal (SSL) genetic interactions from a systematic assay of over 700,000 gene pairs in S.

cerevisiae. Here we describe: a) a comparison of the value of SSL and protein interactions in predicting gene function; b) relationships to other biological networks, including network motifs; and c) the combination of SSL and microarray data to explore the role of transcriptional compensation in *S. cerevisiae*'s robustness to gene loss.

SGA screens identify genetic network interaction among replication (rad27), double-strand break repair (rad52) and nuclear pore (nup133) deficiencies in S. cerevisiae

Sophie Loeillet *, Benoît Palancade[#], Marina Cartron[#], Agnès Thierry[∫], Guy-Franck Richard[∫], Bernard Dujon[∫], Valérie Doye[#x], and Alain Nicolas*[x]

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Previous genetic interaction studies, including a synthetic genetic array (SGA) and synthetic lethality analysis on microarray (SLAM) analysis, showed that the survival of *rad27Δ* cells in *S. cerevisiae* requires several DNA metabolic processes, in particular those mediated by all members of the Rad52-dependent recombinational repair pathway.
We have independently performed a SGA screen of the *rad27Δ* cells against the collection of non-essential yeast genes with variant markers and protocols. It resulted in the identification of an overlapping but not identical set of rad 27*Δ* (Loeillet et al., 2005 DNA Repair, 4, 459). Most notably, we detected ą synthetic lethality affecting the Nup84 nuclear pore subcomplex (nup133Δ, nup120Δ and nup84Δ) in conjunction with the *rad27Δ* mutation. Additional SGA screens showed all the Rad52 group genes are required for the survival of the *nup133Δ* and *nup120Δ* mutants, which are defective in nuclear pore distribution and mRNA export, but not of the *nupı Δ* mutant, which is solely defective in pore distribution.
These data and our own experience in conducting SGA screens will be presented.

Regulated Cell to Cell Variation in a Cell Fate Decision System

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We studied the quantitative behavior and cell-to-cell variability of a prototypical eukaryotic cell fate decision system, the mating pheromone response pathway in yeast. We dissected and measured sources of variation in system output analyzing thousands of individual, genetically identical cells. We found taht only a small portion of total cell-to-

cell variation was caused by random fluctuations in gene transcription and translation during the response ("expression noise"). Instead, variation was dominated by differences in the capacity of individual cells to transmit signals through the pathway ("pathway capacity") and to express genes into proteins ("expression capacity"). Cells with high expression capacity expressed genes at a higher rate and increased in volume more rapidly. In addition, our results revealed two mechanisms that regulated cell-to-cell variation in pathway capacity. First, the pathway MAP kinase Fus3 suppressed variation at high pheromone while the MAP kinase Kss1 enhanced variation at low doses. Second, pathway and expression capacity were negatively correlated, suggesting a compensatory mechanism that allowed cells to respond more precisely to pheromone in the presence of a large variation in expression capacity.

Homeostatic adjustment and metabolic remodeling in glucose-limited yeast cultures.

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We studied the physiological response to glucose limitation in batch and steady-state (chemostat) cultures of Saccharomyces cerevisiae by following global patterns of gene expression. Glucose-limited batch cultures of yeast go through two sequential exponential growth phases, beginning with a largely fermentative phase, followed by an essentially completely aerobic use of residual glucose and evolved ethanol. Judging from the patterns of gene expression, the state of the cells growing at steady state in glucose-limited chemostats corresponds most closely with the state of cells in batch cultures just before they undergo this "diauxic shift." Essentially the same pattern was found between chemostats having a fivefold difference in steady-state growth rate (the lower rate approximating that of the second phase respiratory growth rate in batch cultures). Although in both cases the cells in the chemostat consumed most of the glucose, in neither case did they seem to be metabolizing it primarily through respiration. Although there was some indication of a modest oxidative stress response, the chemostat cultures did not exhibit the massive environmental stress response associated with starvation that also is observed, at least in part, during the diauxic shift in batch cultures. We conclude that despite the theoretical possibility of a switch to fully aerobic metabolism of glucose in the chemostat under conditions of glucose scarcity, homeostatic mechanisms are able to carry out metabolic adjustment as if fermentation of the glucose is the preferred option until the glucose is entirely depleted. These results suggest that some aspect of actual starvation, possibly a component of the stress response, may be required for triggering the metabolic remodeling associated with the diauxic shift.

Dynamics in induced repression of phosphomannose isomerasePMI40 gene of Saccharomyces cerevisiae

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GDP-mannose is the precursor of cell wall biosynthesis in *S. cerevisiae.*To understand the system level role of GDP-mannose, we studied a conditional knock-out strain of the key enzyme in its synthesis; *PMI40*.. The experimental procedure allowed us to study the order of mechanisms the cells launch in order to adjust to a sudden malfunction in the metabolic machinery. We collected 100 samples from the continuous cultivations for 80 hours and measured genome-wide gene expression levels, 10 enzyme activities, and concentrations of 30 intracellular metabolites. In order to carry out this magnitude of experimentations we needed to generate a bioLIMS to handle all the experimentations and generated data. Furthermore we built a sample-taking robot, which automatically took and preserved the samples. A proprieatary software platform, with e.g. workflow and pathway editors, was generated for the in silico part of the work. After normalization and clustering, significantly changed genes and metabolites were searched for enrichment in biological processes, molecular functions, and macromolecular complexes. Further, gene expression levels, metabolite concentrations, and enzyme activities were searched against each other for causality over time. Overall, we focused on thorough analysis of our own data and known database data in order to reward our efforts with knowledge.

At the transcriptome level, repression of *PMI40* activated various stress responses, such as osmotic stress, heat, oxidative stress, nutrient depletion, and DNA damage. Unexpectedly one third of the stress genes were induced even before the repression of *PMI40* had affected the corresponding enzymatic activity or GDP-mannose concentration. Genes involved in response to osmotic stress were well represented in this set, suggesting a role for the Hog1p-MAPK cascade in the initial stress response. Phd1p and Skn7p were identified as high-ranking transcription factors possibly playing a role in the initial regulatory events. Over a longer time frame the repression of *PMI40* led to starvation, as indicated by the induction of genes involved in filamentous growth and mating.

Integrative analysis of the cell sizer network controlling entrance into S phase: role of the nucleo/cytoplasmic localization of Sic1

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The control of cell cycle is a very open question that may gain new strength via a modular systems biology approach (1). The coupling of cell growth to cell division is a universal but poorly understood feature of the cell cycle. In budding yeast a critical cell size (Ps) is required to enter S phase. Ps increases at faster growth rates.
A two threshold mechanism involving the inhibitors of the cyclin dependent kinases Far1 and Sic1 has been reported to control entrance into S phase in budding yeast (2). Further studies have shown that Sic1 localization into the nucleus requires an NLS and that Sic1 facilitates nuclear accumulation of Clb5. Moreover the nucleo/cytoplasmic localization of Sic1 appears to be modulated by carbon source (3).
Taking together all these results a mathematical model of the G_1 to S transition has been constructed and analysed by simulation. The model is shown to accurately predict the behaviour of more than 40 mutants, and the differential dynamics of Sic1 and Clb5 in G_1 cells entering S phase in differential conditions. Algorithms for the simulation of cell population dynamics have been developed. The estimated values of Ps in different growth conditions support the notion that the critical cell size is an emergent property (4) of the described network and that its value is set by few relevant parameters. The perspectives of this line of investigation will be discussed.

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(4) Palsson B. O. et al, "Reconstruction of cellular signalling networks and analysis of their properties", Nature Reviews Molecular Cell Biology, 2005, 6: 99-111.

Enhanced Biomolecular Network Reconstruction via Use of Heterogeneous Data

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One of the central endeavors in Systems Biology is the reverse engineering of cellular networks, which is the first step towards rational prediction and control of the underlying organism behavior. While much empirical knowledge has been gained since the advent of the high-throughput experimental techniques, the task remains challenging due to, among other things, the inherently high structural and functional complexity of most biological molecular reaction pathways. Additionally, such network reconstruction efforts could be further impeded by the broadly heterogeneous nature of data sources, which may include microarray expression, ChIP-chip binding, yeast two-hybrid interaction and other types of observations, as the amount of information coming from any one source is still largely insufficient to enable the unambiguous inference of regulatory network structure directly. To address these challenges, many proposed reconstruction methods have relied on both highly abstracted and largely phenomenological or statistical models as a way of reducing

complexity and increasing predictive power [1-8]. Recently, more biochemically-driven methods, which generally use the reaction modeling techniques of deterministic chemical kinetics to meaningfully constrain target biomolecular pathway properties, have begun to appear [9-12]. For example, one of these methods, NCA (by Liao et al. [12]), utilizes a kinetics-based formalism to deduce a bipartite network control matrix and regulator time-courses from the expression time-series and a set of structural assumptions about the underlying gene regulatory network connectivity pattern. Our new unsupervised network reconstruction method, ENRICH (Enhanced Network Reconstruction via Inference of Connectivity/Control from Heterogeneous data), accrues the benefits of such approaches and is similar in spirit, if not in mathematical algorithm, to the NCA. ENRICH, however, does not impose constraints on the possible network connectivity patterns and is further able to simultaneously integrate the heterogeneous interaction and time-series data, thus increasing their combined predictive power. This allows ENRICH to, among other things, incorporate network structure hypotheses from other reconstruction methods – whether analytical or empirical – and to thus build upon their inferences by deducing additional significant interactions.

We tested ENRICH on *Saccharomyces cerevisiae* cell-cycle microarray data (Spellman et al. [13]) in combination with interaction information in the form of 79 PCR-confirmed ChIP-chip transcription factor bindings (Lee et al. [14]). The algorithm inferred 383 new regulatory connections. For 5 regulators (Gal4, Gcn4, Msn4, Ste12 & Zap1), which had extensive independent gene-regulatory data available, we were able to find experimental evidence for over 54% of the 122 newly deduced regulatory controls, resulting in a 77% estimate for the percentage of all predicted regulatory interactions called correctly by ENRICH. In addition, one of the key uses for the algorithm is to, among other things, predict the interactions of central regulators that are difficult to manipulate genetically or otherwise because they essentially involve – directly or indirectly – many targets and because their concentrations must be tightly controlled or else toxicity arises. Rap1 is one such protein for which our method correctly infers many known regulatory interactions and predicts a number of new ones to be followed up.

In Vivo Identification of Yeast Glycolysis

Gunnar Cedersund

Linköpings Universitet, Sweden

Yeast glycolysis is one of the most well-characterized biological systems and it is also a system for which there exists a significant amount of data. Yeast glycolysis therefore serves as an excellent test case for many methods and promises within systems biology. In recent years quite a few large scale models of yeast glycolysis have been developed. An example of such a model is given by Hynne et al (Biophys. Chem., 2001, 94, 121-63), which also captures oscillations obtained in CSTR experiments. These models are typically based on in vitro estimates of the kinetic parameters followed by parameter adjustments to make the model as a whole reproduce the in vivo observations. However, the information contained in the available in vivo data is not sufficient to uniquely

determine the kinetic parameters, i.e., the models are not identifiable. One can therefore not claim to have in vivo estimates of the kinetic parameters, or of the system features (like overall time-constants or control coefficients), in these models. We report two methods that allow for such in vivo estimations.

The first method provides in vivo estimation of the kinetic parameters within a single reaction. It can be applied to reactions where direct measurements of all involved substrates, products, modifiers, and flux are available. This is experimentally possible, e.g., for the phosphoglucoisomerase reaction. We show that the parameter estimation problem is then often reduced to solving an overdetermined set of linear equations. Furthermore, we show how to robustly solve these equations in the presence of noise.

The second method is suited for in vivo estimation of system features. It starts by applying model reduction techniques to a gray-box model to obtain an identifiable core model. The parameters of the core model are then uniquely estimated from the data and the features of the core model are translated back to the original gray-box model. The end result is a combination of the two models and we therefore denote this second approach core-box modeling.

In the final part of the talk we use a newly developed method to reveal what generates temporal oscillations, e.g., in the Hynne et al model. We find that the oscillating core consists of the feedback from the ATP-consuming parts back to the ATP producing phosphofructokinase, which is also allosterically regulated by ATP. This result agrees well with recent conclusions drawn by another method from phase-relation data. This last analysis also serves as an example of what kind of analyzes can be done, once a reliable in vivo model is developed.

Abstracts for posters

Metabolome meets Transcriptome: Fast Dynamics Reveal Correlation between Metabolome and Transcriptome

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Biological system is such a complex system. Inside the cell, hundreds of molecules interact with each other and thousands of reactions occur simultaneously. To achieve a better understanding of what happens inside the cell a dynamic inter-'omics' analysis: transcriptome, proteome and metabolome is necessary. So far it is always assumed that cell regulation in the transcriptome and proteome level is much slower than metabolome level. Thus within the first few minutes following the pulse perturbation the enzyme concentration is assumed constant and the observed responses can be attributed to kinetic interactions at the metabolome level. However, the assumption is made without knowing the exact temporal expression program or the rate of protein synthesis since no quantitative transcriptome and proteome approach on such short time scale have ever been achieved. This study, for the first time, presents the combined metabolite and genome wide expression analysis of the fast dynamics *S. cerevisiae* response upon glucose pulse in a steady state glucose limited chemostat culture in the time frame of 360 seconds.

The study was performed in a Stimulus Response Technology experimental set up (Lange *et al.*, 2001; Theobald *et al.*, 1997), in which a glucose pulse was introduced into a steady state chemostat culture and subsequently samples were taken for intracellular metabolite (nucleotides, glycolytic and TCA cycle intermediates), extracellular metabolite (glucose, ethanol, acetate and glycerol) and transcript analysis (genome wide analysis by Affimetrix microarray). The results provide new insight in the chronological events between the metabolic and the transcriptional response and show a biological significant correlation between metabolome and transcriptome with respect to energy and nucleotides regulation.

References:

Lange HC, Eman M, van Zuijlen G, Visser D, van Dam JC, Frank J, de Mattos MJT, Heijnen JJ. 2001. Improved rapid sampling for in vivo kinetics of intracellular metabolites in Saccharomyces cerevisiae. Biotechnology and Bioengineering. 75(4):406-415.

Theobald U, Mailinger W, Baltes M, Rizzi M, Reuss M. 1997. In vivo analysis of metabolic dynamics in *Saccharomyces cerevisiae* : I. Experimental observations. Biotechnology and Bioengineering. 55(2):305-316.

Genome-wide Translational Control in Fission Yeast

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We are interested in global roles of translational regulation during the cell cycle and in response to environmental factors. To obtain translational profiles for all mRNAs, polysome preparations are separated according to their size using a sucrose gradient, and the mRNAs in each fraction (or pools of fractions) are identified and quantified with DNA microarrays (e.g. [1]).
Starting with exponentially growing cells, we analyzed 13 polysome fractions using DNA microarrays containing elements for all known and predicted genes of fission yeast (*Schizosaccharomyces pombe*). This approach provided data on average numbers of associated ribosomes for most transcripts. A preliminary integration with data on mRNA steady-state levels revealed a strong bias: the most abundant transcripts seem to be associated with many more ribosomes than less abundant transcripts, although ribosome density seems to only correlate weakly with transcript levels. We are testing whether this trend depends on either transcription or mRNA decay rates. Translational profiling of cells in different cell-cycle stages or subjected to various perturbations will provide a genome-wide view of translational regulation in fission yeast, complementing our expression profiling data. Preliminary results of these studies will be presented.

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Space in Systems Biology of Signaling Pathways

Kouichi Takahashi

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Extracellular signals captured by receptor proteins on the cell surface are transmitted inward to control gene expression. In vivo protein diffusion speed is significantly slower than in saline solutions, and is comparable to response timescales of signaling pathways [1], implying at least some of the biochemical reactions in the pathways are limited by diffusion. Diffusion-limited reaction can amplify effects of noise [2] and, when coupled with localized proteins, can also affect signaling outcomes [3]. Furthermore, extremely high protein concentrations (50-400 mg/ml) in the intracellular space, commonly called molecular crowding, can magnify the spatial effects [4].
This presentation will discuss (1) simulation methods that can be used to investigate the spatial effects, (2) how those computational frameworks can represent intracellular molecular crowding either in implicit, semiexplicit or fully explicit way [5], and (3) feasible couplings of such computer modeling approaches with in vivo protein mobility

measurement techniques such as fluorescence correlation spectroscopy. This study is part of the Molecular Sciences Institute's Alpha project, which aims to enable precise and quantitative prediction of the mating pheromone response pathway of S. Cerevisiae.

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System biology approach in studies of SNF1 kinase complex functions in regulatory networks affecting cellular metabolism

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Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark

Snf1 protein kinase and its regulatory subunit Snf4 from *Saccharomyces cerevisiae* have highly conserved mammalian AMP-activated protein kinase (AMPK) analogs. Lately AMPK have become one of the targets for specific drug creation against diabetes, obesity or other metabolic disorders. Therefore, the aims of this study were to elucidate the metabolic network affected by the SNF1 complex and to draw parallels with AMPK regulatory functions in mammalian cells.

The *S. cerevisiae Δsnf1*, *Δsnf4*, and *Δsnf1Δsnf4* strains were characterized in glucose and galactose batch fermentations. Results showed that after glucose depletion, galactose repression lasted 5, 13, 16 and 50 hours for the reference, *Δsnf1*, *Δsnf4*, and *Δsnf1Δsnf4* strains respectively. Moreover, specific growth rates on galactose were lower in glucose repression mutants. To analyze gene expression, yeast cells were pre-grown in glucose limited chemostats. Data showed that not only glucose repression but also other metabolic pathways such as glycogen, lipid and amino acid metabolism were strongly affected. A metabolic model integrated transcription data analysis indicated that SNF1 complex affected cross-points between central carbon metabolism and pathways related to stress response, nitrogen metabolism and cell energy balance. The *Δsnf1Δsnf4* strain had the most different transcriptional profile with abundant changes in amino acid metabolism.

Data indicated that partial suppression of constitutive repression or leaky repression occurred in single deletion mutants, but a more complete repression took place in the double deletion mutant. Previously not described targets of SNF1 complex were pointed

out using advanced transcriptome analysis tools. This might lead to elucidating more overlaps with mammalian AMPK functioning and new targets for treatment of various metabolic disorders.

Identification of the network controlling the G1/S transition in yeast: a key role for subcellular localization of Sic1

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Department of Biotechnology and Biosciences, University of Milano Bicocca Milano Italy,

Systems biology aims to describe the structure of a biological system (i.e. its determinant molecular components and their wiring in a regulatory network) and to predict its dynamics under a spectrum of different conditions[1], the goal being to achieve a comprehensive body of knowledge of biological systems, solidly grounded at the molecular level[2]. In the absence of a complete analytical knowledge of the components of a system, cellular processes can be conveniently considered as modules, subsystems of interacting molecules such as proteins, DNA, RNA and metabolites that perform a given function in a way largely independent from the context[3]. Modules are linked by governing interactions that follow general design principles that are well known in engineering, such as switch, threshold control, positive and negative feedbacks, amplification, error correction, etc. The components of a module and their interactions can be identified following iterative application of the **4M** Strategy (quoted in ref. 4) - **M**ining of literature and data banks - **M**anipulation of the module structure and function - **M**easurement of all putative regulatory components - **M**odeling and simulation.
We are applying this approach to the study of the G1/S transition of the budding yeast *Saccharomyces cerevisiae*[5]. The cyclin dependent kinase inhibitor Sic1 and the cyclin Clb5 are essential regulators of the cyclin dependent kinase Cdc28 during the G1 to S transition in budding yeast. We recently showed that Sic1 is involved in carbon source modulation of the critical cell size required to enter S phase[6]. Here we show that the amount and sub-cellular localization of Sic1 are carbon source-modulated. Nuclear localization of Sic1 depends upon a bipartite localization sequence and is essential for its role *in vivo*. Similarly to Cip/Kip proteins – Sic1 mammalian counterparts – Sic1 facilitates nuclear accumulation of its cognate cyclin, as shown by cytoplasmic accumulation of Clb5 upon switching off expression of the *SIC1* gene. These findings have been incorporated in the network controlling entrance into S phase that for the first time accounts for nucleo-cytoplasmic shuttling of Sic1 and Sic1 complexes. Matematical modelling and simulation of the network are reported in an accompanying report[7].

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High-throughput and quantitative metabolome analysis – evaluation of extraction methods

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In the context of designing cell factories, it is desirable to obtain quantitative data for a large number of state variables within the cell. Metabolome analysis is an important, yet relatively unexploited tool for this purpose. The use of high-throughput metabolome analysis in metabolic engineering has been limited by the lack of global approaches to quantitatively identify large families of intracellular and extracellular metabolites. Specifically, sample preparation is still considered to be a limiting step. The diverse chemical and physical nature of metabolite structures gives rise to considerable experimental challenges in extracting diverse molecular families. We have explored five different strategies for their ability to extract a significant number of metabolite families from the yeast *Saccharomyces cerevisiae.* Mass spectrometry, which is increasingly occupying a central role in metabolome analysis, was used to characterize reproducibility for target analysis of pre-defined metabolites and metabolite profiling. Specifically, gas chromatography coupled to mass spectrometry (GC-MS) and direct-infusion mass spectrometry were employed. We will report a rapid, robust, and consistent method that can be applied to the identification of a large number of intracellular metabolites from this yeast. In addition, we will comment on the use of this method in a more general framework for the integration of quantitative metabolome analysis with transcriptome studies for understanding design principles of the cell.

Mathematical Modeling of the G1/S Transition in Yeast Cell Cycle

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The eukaryotic cell cycle is the repeated sequence of events accompanying the division of a cell into daughter cells. It is divided into four phases: G_1, S, G_2 and M. Passage through the cell cycle is strictly regulated by the periodic synthesis and destruction of *cyclins* that bind and activate *cyclin-dependent kinases* (CDKs). The levels of cyclins rise and fall during the stages of the cell cycle. The levels of CDKs appear to remain constant during cell cycle, but individual molecules are either unbound or bound to cyclins. *Cyclin-dependent kinase inhibitors* (CKI) contribute to cell cycle control by coordinating internal and external signals and blocking proliferation at several key checkpoints.

Budding yeast is a well studied model organism for modeling of cell cycle[1,2]. A major regulating event is the G_1 to S transition, in which the cell has to reach a critical size in

order to be able to enter into S phase. Work from one of our laboratories has allowed identifying the regulatory network controlling the entrance into S phase, which involves the CKI Far1 and Sic1[3]. Besides, more recent work[4] has allowed dissecting the role of the nuclear/cytoplasmic localization in the regulation of the activity of Sic1, whose functional homology with the CKI p27^{Kip1} of mammalian cells has been described[5].
Taking together own results and data from the literature, we have constructed a model for the G_1 to S transition. The graphical model has been implemented by a set of ordinary differential equations (ODEs)[6]. These equations describe the temporal change of the concentrations of the involved proteins and complexes. Moreover, the mathematical model accounts for the cell growth during the G_1 phase as well as for the localization of components in different cell compartments (cytosol or nucleus).
The model allows simulating the dynamics of the G_1 to S transition in various growth conditions and in response to different signaling pathways. Sensitivity analysis is used to estimate the influence of parameter values on temporal behavior of key components and to show the relevance of the nuclear/cytoplasmic localization of Sic1[7], modulated by carbon source, on the dynamics of the G_1 to S transition, which has been neglected by earlier models of the cell cycle.

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Characterization of Glucose Repression and Longevity Transcriptional Regulatory Networks in Saccharomyces cerevisiae.

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Elucidating the structure and function of transcriptional regulatory networks has shed new insight into complex biological phenotypes, but is methodologically complicated by the complexity inherent in such networks. Towards these ends, we previously demonstrated a novel experimental and computational

method for reverse-engineering a first-order model of regulatory interactions in the SOS network of *Escherichia coli* (2), and here apply a similar approach to a ten-gene network involved with glucose repression and longevity in *Saccharomyces cerevisiae*.
S. cerevisiae strains were created which allowed for tetracycline-inducible overexpression (1) of each of the ten genes in the network studied, which included the glucose-repression regulator kinase *SNF1*, its associated protein complex subunits *SNF4* and *SIP2*, downstream transcription factors *CAT8*, *SIP4*, *MIG1*, and *MED8*, and the glucose-repressed enzymes *HXK2*, *FBP1*, and *SUC2* (3, 4). mRNA expression was assayed by quantitative RT-PCR in response to single-gene perturbations relative to control at a steady state, and the normalized expression ratios were used to construct a network model as a system of ordinary differential equations. We then employed an algorithm (NIR, or Network Inference by Regression) to learn the coefficients by multiple regression that minimized the least-squares error, providing a quantitative description of the sign (negative, positive, no interaction) and strength of regulatory influences of one network gene upon another.
The recovered network model identified many of the known interactions between genes, as well as a large number of interactions previously uncharacterized. These novel predicted interactions were tested using gene deletions and promoter/reporter gene fusions to determine if the absence of the regulating gene in question affected expression of a *lacZ* reporter gene under control of the putative target gene's promoter. Chromatin Immunoprecipitation (ChIP) experiments were performed to examine whether newly predicted regulatory interactions occurred by direct transcription factor promoter binding. These results confirmed the majority of interactions predicted by the network inference strategy, indicating a previously uncharacterized high degree of connectivity and the prevalence of positive and negative feedback in transcriptional control of the Snf1 pathway.
To further characterize the role of feedback suggested by this analysis, we modeled the dynamics of a two-gene subnetwork (*SNF1*, *SIP2*) by a system of delay differential equations. The model is based on mass-action kinetics, and accounts for regulation of gene expression, protein complex formation, modification and transport between the nucleus and cytoplasm (5). Specifically, Snf1 shuttles to the nucleus in low glucose, causing delayed upregulation of Sip2, a cytoplasmic protein. Sip2 forms a complex with Snf1, sequestering it to the cytoplasm and inhibiting further transport to the nucleus. In summary, *SIP2* and *SNF1* form a two-gene negative feedback loop, which exhibits interesting dynamics following transition from high to low glucose conditions.

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PathwayLab – A Customizable Modeling and Simulation Tool

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PathwayLab is a software tool for modeling, simulation, and information management of biochemical reaction networks. The software facilitates the modeling process by an easy to use drag-and-drop graphical user interface where reaction networks quickly are assembled using ready made or user defined modeling objects. An important objective

when designing the tool was to provide a framework that could support several exiting and future graphical formalisms for representing biochemical networks - easily customizable by the user. At the same time the objects used for assembling the graphical networks should constitute containers for mathematical equations and easily map to complete mathematical representations of the networks such as the reaction rate equations or code for applying the stochastic simulation algorithm. In PathwayLab we have succeeded in condensing the number of abstract model object classes to the basic objects: entities, transformations, controls, and locations. The current functionality of PathwayLab includes but is not limited to transient simulation, steady-state analysis, metabolic control analysis, export of models to Mathematica, Matlab, and SBML, parametric scans, time-course and phase plane plots, import of tabulated data to be interpolated during simulation, export of simulation data, and data base connectivity. All put together to make PathwayLab a valuable addition to the toolbox of the systems biology community.

Multi-scale Genetic Network Inference Based on Time Series Expression Profiles

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Gene expression data are noisy, large scale and with groups of genes co-regulated.
Clustering is widely used to group genes with similar pattern. Cluster centers can be used to infer the genetic networks among these groups of genes. Multi-scale fuzzy kmeans clustering algorithm can uncover groups of co-regulated genes with different degrees of co-regulation and capture the networks in different levels of detail.
Time series expression profiles provide dynamic information for inferring gene regulatory relationships. Gene expression regulation can happen in specific time periods and conditions instead of over the whole expression profile. Identifying the transient interactions, feedback loops and telling direct interactions from indirect interactions are among major challenges of genetic network inference. This work uses short-time correlation to capture transient interactions and show how network structures dynamically change over time. Time correlation can also estimate the time delay and direction of causality in the inferred network. Partial correlation and d-separation theory can be combined to differentiate the direct and indirect interactions and identify feedback loops.
This work integrates multi-scale clustering and short-time correlation to estimate regulatory networks at different time scales and degrees of coregulation. The algorithm was evaluated using yeast cell cycle data. The results give the networks at different levels of detail, and reflect most interactions previously identified by genome-wide location analysis[3]. Significant regulatory sequence motifs were identified based on the clusters at different levels and match literature results. The algorithm successfully identified the yeast cell cycle development stages, cell cycle loop and negative feedback loops, and

indicated how networks dynamically change over time and in which time intervals the interactions happen.

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BioRica: integrating continuous and discrete models in a single framework

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BioRica is a framework that offers a unified view of various modeling approaches for biological systems (e.g. differential equations, Markov processes etc.).
Living cells are complex systems consisting of distinct but interacting components. Adequately modeling this interacting organization is key to understanding their behavior, that is being able to analyze how these components interact in order to produce the observed emerging property.[1]
It is now well accepted that the most precise units for modeling biological processes are the kinetic reactions occurring in, or between, the cell compartments. However, it is also recognized that some part of these processes can be described using higher order behavioral units without a significant loss of precision, while being easier to handle. Description, studying and verification of such models are now commonly made using computer simulation and formal mathematics by biologists. For this approach, two major model classes arose. On one hand, some biological phenomena can be captured within a discrete or state/transition paradigm, by exhibiting various causality chains between biological events and objects. Moreover, the use of probabilities and explicit timing behavior allows for great expressivity in these models, while sustaining natural intelligibility. On the other hand, from a computational point of view, the order of magnitude of the vast majority of processes leads us to consider another (and most widely used) paradigm in systems biology, that is Ordinary Differential Equations. This so called continuous paradigm is often used to fully describe the behavior of a particular cell function. Rather than considering these two paradigms as antagonistic, we aim at integrating them in a single modeling language with a unified semantics. Such an approach allows considering in one and the same model small and large scale interactions, timed and untimed behavior, precise or probabilistic descriptions, all the while taking into account the modularity observed in biological interactions.
This goal is achieved through the S-AltaRica DataFlow language, a formal modeling language that describes a given system in terms of interacting components. The AltaRica

language[2] allows textual and graphical description of such modular systems exhibiting discrete behavior by using timed cause and effect rules. Each rule can also have associated discrete and/or continuous probabilities, allowing for the description of a large set of behaviors within a single model. The semantics of an S-AltaRica DataFlow model is given in terms of stochastic mode automata.[3, 4] Furthermore, an automatic translation of ODE systems into AltaRica rules allows direct import of large scale systems, with a reasonably small overhead compared to pure numerical integration. Such a translation makes possible easy inclusion of existing SBML and Cellerator models into a AltaRica components. The description of the various components of a model uses an object oriented approach, permitting constructs such as synchronization and data sharing between components An efficient simulator BioRica toolkit provides a random simulation framework for S-AltaRica DataFlow models. The toolkit relies on the gcc compiler family to generate optimized automatic or interactive simulators, tailored to each model1 and based on a discrete event approach[5]. The simulation results are then processed by statistical analysis tools. We will illustrate the use of BioRica on small to middle-sized models. A demonstration of the tool will be available during the poster session.

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Towards Computational Analysis of Metabolic Profiling Data for Protein Function Prediction

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We are developing computational methods for the identification of system level 'functional signals' which can be captured from metabolomics data and confirmed by other heterogeneous data sources. **Objective:** The objective of the current work is to develop computational approaches for the analysis of metabolic profiling data and physiological data, like growth profiles etc. combined with complimentary and confirmatory results from heterogeneous data sources like Transcriptomics data, to identify functional associations among genes.
Results: Here we present, the results from a preliminary statistical study of a selected set of gene knockout mutants involved in the regulation of central carbon metabolism in *S.*

cerevisiae, using the (i) Partition Around Medoids (PAM) and Hierarchical Clustering (HC) methods for finding the optimal number of gene clusters using physiological yield data (PHY) and (ii) Euclidean distance based on co-response profiles (CP), as a means of confirming the functional associations found using the PAM and HC methods. The knockout strains are exposed to different carbon sources namely Glucose, Fructose and Galactose, to identify the differential effects of knocked-out genes under different environment conditions. We found that 35% of gene pairs which showed CP correlation above 0.7, were also assigned to the same cluster or adjacent cluster based on PHY data.
We identified YIL107C (PFK26) and YIL154C (IMP2) as one gene pair having functional association and by studying the literature we propose that IMP2 is involved in transcriptional regulation and requires PFK2 activity for the process. Further studies are needed for the confirmation of this 'functional signal'. YIL107C (PFK26) and YOL136C (PFK27) are another pair of genes with a high co-response value and indeed, the genes are iso-enzymes catalyzing essentially the same reaction, though PFK26 shows a higher activity than PFK27. Systematic analysis of CP and PHY data was performed using R, as follows: (i) Calculation of pair-wise Euclidean distance for all ORFs based on PHY data, (ii) Generation of a symmetric matrix with gene pair CP correlation values. A Fischer test on these data gave p-values below 0.05 for all three carbon sources, thereby confirming the hypothesis that those gene pairs which show close association in physiological yield also show high correlation in their transcript co-response profile. Further, we are working towards combining heterogeneous data sources like Mass Isotopomer Distribution(2) profiles and in-silico flux estimates for the development of a framework for protein function prediction exploiting metabolomics data.

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Characterization of Cellular Pathways Using Time-Varying Signals

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The use of traditional tools for the discovery and characterization of biological systems has resulted in a wealth of biological knowledge. Unfortunately, only a small portion of the biological world is well-understood to date, and the study of the rest remains a daunting task. This work involves using time-varying stimuli in order to more rapidly interrogate and characterize signaling pathways. The time-dependent stimulation of a signaling pathway can be used in conjunction with a model of the pathway to efficiently evaluate and test hypotheses. We are developing this technology using the yeast pheromone signal transduction pathway as a model system. The time-varying stimuli will be applied to the yeast cells via a novel microfluidic device, and the pathway output will be measured via various fluorescent reporters. The output of the pathway can then be compared to the output from a computational model of the pathway in order to test hypotheses and constrain our knowledge of the pathway. Initial work shows that a

computational model can be used to identify stimuli time-courses that increase the parameter sensitivity, meaning that corresponding experiments could potentially be much more informative.

In vivo robustness/sensitivity analysis of cell division cycle related genes in S. cerevisiae.

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Robustness is an emergent property of complex, evolvable systems such as the cell (1). Major experimental efforts have elucidated the molecular events of yeast cell proliferation in a resolution high enough to develop a kinetic *in silico* system (2). Sensitivity analysis using these models predict robust components, but how do the *in silico* predictions fair against *in vivo* results? And what can be elucidated by knowing it? In an attempt to answer these questions, we performed a genetic system-wide robustness/sensitivity analyses on the cell division cycle. Using 'Genetic tug-of-war' method, upper limit of gene dosage of 30 cell division cycle genes was determined. In conjunction we developed a comprehensive cell division molecular interaction map to visualise and model the results, which suggests B-type cyclin regulations are sensitive processes. Although the majority of experimental data were inconsistent with the *in silico* results, we propose possible improvements of the current model using these data to identify novel regulatory components and loops.

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