Saccharomyces cerevisiae, glucose is the preferred sugar that is metabolized and is also a stimulus that controls the expression level of some quarter of the yeast's genes. Interestingly, yeast can measure the level of extracellular glucose through its various sensors, but not directly how much glucose it's importing. Two of these sensors, Snf3 and Rgt2, detect the concentration of extracellular glucose and accordingly regulate the transcription of the passive hexose transporters (HXTs) that are essential for glucose uptake in yeast. Here, we show that when the transcription of HXTs is controlled independently of the two sensors, surprising behaviors in the cell's growth rate are observed. In particular, both increase in glucose uptake rate (GUR) and the extracellular glucose concentration can each lead to substantial decrease in cell's growth rate. We therefore show that the growth rate of the cell in batch cultures is not just a function of how much glucose the cell eats, but also depends on how much glucose the cell senses outside. We attribute these growth rate behaviors to an imbalance between availability and consumption of glucose. By studying a *snf3Δ*, *rgt2Δ* mutant, the two sensors are shown to have an additional role in determining the growth rate than just through the transcriptional control of the HXTs. Furthermore, we have discovered that when just one of the main hexose transporters (HXT1~4, and HXT6) is present in a cell, glucose-sensitive post-transcriptional controls of that HXT other than the known endocytosis mechanism exist. Finally, we use an analytical model to reveal constraints placed on the synthesis of each HXTs to ensure proper scaling of GUR with extracellular glucose concentration.

1548-Pos Board B392

Modeling Intercellular MAPK Signaling in an Epithelial Wound Healing Assay

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Recent experiments in epithelial wound healing have demonstrated the necessity of MAPK activation for coordinated cell movement after damage. This MAPK activity is characterized by two wave-like phenomena. One MAPK ''rebounding wave'' that originates immediately after injury, propagates deep into the cell layer, and then regresses back to the wound interface. The second MAPK wave is a slow developing, sustained wave that propagates from the wound interface. Experimentalists have suggested that the first wave is originated by reactive oxygen species (ROS) generated at the time of injury. We develop a mechanistic diffusion-convection model that produces the observed behavior by taking advantage of the coupling between ligand (e.g. EGF) and ROS species in the activation of the MAPK cascade. In our model, the second wave is initiated, and sustained by the stresses induced by the slow cell movement toward the injury. We explore the bi-stability of the model in connection with the bi-stability of the MAPK cascade. In particular, we look for traveling wave solutions of the model and their properties under various regimes.

1549-Pos Board B393

Modeling cAMP-cGMP Crosstalk in the Cardiac Myocyte

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While the role of nitric oxide (NO) in regulating cardiac function through vascular smooth muscle relaxation has been characterized over the past 15 years, NO's effects in the cardiac myocyte have yet to be resolved. The addition of NO to these cells has been reported to cause a biphasic response; NO either increases or decreases cardiac contractility depending on its concentration. Proposed mechanisms for this response include a number of factors, ranging from direct nitrosylation of the ryanodine receptor by NO to modulation of the beta-adrenergic signaling pathway by NO-induced cGMP. This latter interaction is supported by experimental data showing a concomitant biphasic response of the L-type calcium current.

In order to elucidate the mechanisms underlying the biphasic response of the L-type calcium current to NO, we have developed a model combining descriptions of cAMP production via the beta-adrenergic signaling pathway and cGMP production via a NO signaling pathway. The cAMP-cGMP crosstalk model couples the production of cGMP by guanylyl cyclase to the beta-adrenergic signaling pathway via cGMP-activated and cGMP-inhibited cAMP phosphodiesterases (PDEs.) Integrative regulation of cAMP concentration will ultimately regulate the L-type calcium current, via altered activation of protein kinase A.

We hypothesized that the opposing behavior of these two cGMP-regulated cAMP PDEs leads to the biphasic effects on L-type calcium current seen experimentally.

To test this hypothesis, a model was formulated from existing models describing cGMP synthesis and beta-adrenergic control of L-type calcium current. These two pathway models were coupled using enzyme kinetic data describing the PDEs. Simulations from the model combining these two pathways show that the interplay between these two cGMP-regulated cAMP PDEs gives rise to the biphasic response of the L-type calcium current. Supported by R33HL87345.

1550-Pos Board B394

Cdc14-release Oscillation is Separable from Cell-cycle Progression, and Modulated by Clb-Cdk

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A free-running Cyclin oscillator suggests that the oscillation of Cyclin dependent kinase(Cdk) activity can lead to periodicity of cell-cycle events. In budding yeast S. cerevisiae mitotic Cyclin activity is primarily antagonised by the phosphatase Cdc14 which is released from nucleolus in late mitosis and becomes active to promote exit from mitosis. We devised a quantitative assay to study the effect of B-Cyclin on Cdc14 localization. By introducing physiological concentration of nondegradable Clb2-kd into the cell, we found that Cdc14-localization status became oscillating and uncoupled from cell-cycle progression. The frequency of this oscillation is controlled by Clb2-kd concentration, and saturated at twice the frequency of a normal cell-cycle. This observation suggests that cell-cycle progression may be controlled synergistically by kinase and phosphatase oscillators. We proposed a model for the cell-cycle-independent Cdc14 oscillation being a negative feedback oscillator involving the activation of APC-Cdh1 by Cdc14 and the subsequent degradation of polo kinase.

1551-Pos Board B395

Modeling Extrinsic Apoptosis Regulatory Network Pathways Using A Rules-based Framework

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We describe a systems approach to combine mathematical modeling and experimental measurement in the study of apoptosis in mammalian cells. The apoptotic signal is an important all-or-nothing mechanism which must be tightly regulated in the cell. Our focus will be on the role of pro and anti-apoptotic proteins in the extrinsic apoptosis signaling pathway leading to the formation of pores at the mitochondrial membrane by BAK and BAX proteins. This network is a prototypical cue-signal-response-feedback pathway of high biomedical importance. Construction of mathematical signal transduction models that recapitulate key features of signaling pathways as they exist in cells is currently very difficult, in large part because few tools are available to assemble, validate and update large dynamical models. We aim to implement novel methodologies based on ''rules-based'' techniques to allow for a flexible treatment of this complex network model. The calibration of such a model and application to ongoing experimental work in our laboratory is an important aspect of this work. We report our ongoing work on this subject paying particular attention to the rules-based building framework, the calibration steps and the use of experimental data for model calibration and validation.

1552-Pos Board B396

A Graded Response of a Transcription Factor to Increasing Doses of External Stimuli: A Thermodynamic Framework Describing the Behavior of NF-kB

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A paradigm in transcriptional regulation is that a graded increase in transcription factor (TF) concentration is ''digitally'' translated into an on/off transcriptional response by means of cooperative TF binding to adjacent DNA binding sites. Such paradigm stems from the analysis of TFs operating in developmental processes, notably embryonic segmentation, that require the definition of sharp borders separating different body regions.

Here we show that NF-kB, a key TF responsible for the expression of genes implicated in the inflammatory and immune responses, is an ''analogical'' transcriptional regulator. We demonstrate that increasing doses of inflammatory stimuli lead to gradually increasing concentrations of NF-kB in the cell nucleus, which in turn are translated into gradually increasing levels of transcriptional activity of NF-kB target genes. Differently to what observed in developmental systems, we show that the number of NF-kB binding sites in

the promoter regions control the sensitivity of transcriptional induction as a function of NF-kB concentration.
By using a combination of quantitative biochemical measurements, we show that the NF-kB nuclear concentration is always lower than the dissociation constant for NF-kB binding to specific sites on DNA. We integrate these measurements in a minimal model of a promoter containing multiple sites, based on the thermodynamics of the one-dimensional Ising model. We demonstrate that in the measured NF-kB concentration range the model accounts for the observed analogical transcriptional response under the assumptions that NF-kB binding to adjacent sites is not cooperative, and that bound NF-kB molecules recruit the transcriptional machinery in an additive fashion, each NF-kB interacting weakly but additively with the Pol II complex.

1553-Pos Board B397
Information Processing In Single Yeast Cells: Homogeneous Signal Transduction Result In Heterogeneous Gene Expression
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How cells sense their environment using signal transduction pathways and respond to environmental changes by regulating gene expression is a key problem in systems biology. The mitogen-activated protein kinase (MAPK) pathways, which are evolutionarily conserved from yeast to mammals, provide an excellent model to study how signal transduction is coupled to gene expression. Our research focus on the high-osmolarity glycerol (HOG) MAPK pathway in single, Saccharomyces cerevisiae yeast cells. During the last few decades, the components and regulatory network of this pathway have been elucidated via genetic and biochemical assays performed on large populations of yeast cells. However, surprisingly little is known about the detailed coupling dynamics of signal transduction and gene expression in individual cells. After osmotic shock, homogeneous Hog1 kinase dynamics were measured in all cells. In the subsequent gene expression of STL1, a gene that encodes for a glycerol proton symporter of the plasma membrane, we observed that one subpopulation of cells exhibits no gene expression at all (OFF-population), whereas another subpopulation of cells show gene expression over a wide range of expression levels (ON-population). Further, the ratio of the two subpopulations of cells remained constant despite changes in osmolyte concentration from 0.3 M to 0.6 M NaCl. To identify the origin of the bi-modality in gene expression, we over expressed specific transcription factors that regulate STL1 - gene expression. After over expression of one specific transcription factor, we observed a mono modal gene expression distribution for STL1. Furthermore, single cell time-lapse experiments, indicate that switching between gene expression levels after subsequent osmotic shocks was random and uncorrelated. These results indicate, that at least one transcription factor is responsible for the bi-modality and stochasticity in gene expression.

1554-Pos Board B398
Negative Feedback Speeds Transcriptional Response-Time In Human Cytomegalovirus
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Upon infection of a cell, viruses initiate a complex gene-expression cascade that may result in productive/lytic infection, abortive infection, persistence, or latency depending on the state, cell-type, and environment of that particular cell. However, the regulatory networks underlying these diverse viral lifecycle fates are typically studied using experiments that are averaged over cell populations, potentially masking the dynamic behavior in individual cells. To understand how these diverse viral lifecycle fates are regulated at the single-cell level, we present a framework for quantitatively determining viral expression dynamics and regulatory circuit architectures in individual living cells using a simplified model of a herpesvirus signal transduction module: the human cytomegalovirus (HCMV) Major Immediate Early (MIE) circuit. The system utilizes time-lapse fluorescence video-microscopy of cells infected with recombinant viruses expressing fluorescent gene-products and quantitative modeling to analyze the resulting single-cell data. The combined computational-experimental approach revealed two previously unseen transient signal-processing characteristics of the MIE circuit: (i) negative feedback within the MIE circuit, counter-intuitively, speeds the gene-expression response-time of the essential viral transactivator gene-product IE2-86 (ii) transcriptional activators implicated in reactivation from latency (e.g. trichostatin A) generate a pulse in IE2-86 gene-expression in single-cells. As predicted by the model, mutational deletion of the MIE negative-feedback loop eliminated both the IE2-86 pulse and the accelerated response-time during viral infection. We propose that the negative-feedback architecture of the HCMV MIE circuit may allow the virus to respond quickly to external signals and outpace cellular innate defenses at the single-cell level.

1555-Pos Board B399
Pitchfork And Hopf Bifurcations In Stochastic Regulatory Networks With Delayed Feedback
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The bifurcation diagram of a model nonlinear Langevin equation appropriate for delayed feedback loops in regulatory networks is obtained. We show that under parametric fluctuation the bifurcation remains sharp, both in the ranges of direct and oscillatory bifurcation. At threshold, the stationary distribution function becomes a power law in the dynamical variable. The combined effect of delay and stochasticity leads to a shift in the location of the bifurcation threshold relative to the deterministic limit of no fluctuation. The shift scales linearly with the noise intensity.

1556-Pos Board B400
Power-Law Scaling in Protein Synthesis of a Stochastic Regulon-An Experimental Study
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We investigate the protein expression pattern of the lamB gene in Escherichia coli bacterium. The gene product LamB is an important membrane protein for maltose transport into cells but it is also exploited by bacteriophage lambda for infection. Using a dual-colored phage labeling technique, we find that the LamB receptor distribution p(n) has a majority population with average receptor number n~500 and a minority population at small n. This small-n distribution is scale invariant with p(n)~ n̂α. A power law is also observed when LamB expression is chemically repressed by growing the bacteria in a glucose medium. We propose a heuristic model which can account qualitatively for our observations.

1557-Pos Board B401
Exploiting stochastic gene expression to infer promoter regulatory mechanisms: The Moment Analysis Method
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We introduce the moment analysis method for single cell gene expression data. This method exploits higher order moments (greater than two) of steady state fluorescence distributions from genetically identical cells expressing fluorescent proteins to make inferences about the regulatory mechanism of expression at the promoter level. We show that moment analysis can discriminate between mechanisms with a different number of kinetically relevant promoter states, characterized by the transcriptional rate at each state. For instance, we can distinguish between promoters that transcribe genes at a constant rate and those that switch between active and inactive states of transcription. We can also estimate values for the parameters that define the mechanism, such as the transcription rate, the translation efficiency, and the rates at which the promoter switches between different states. We apply this method to analyze gene expression data from the Ptet07 promoter in yeast. In our experiments this promoter is placed at different genomic locations within the yeast chromosome, some of them in silenced and others in open chromatin regions. Our analysis indicates that when we place the promoter-gene system at an open chromatin location, the promoter is well modeled by a single state mechanism, in which the gene is transcribed at a constant rate. Analysis of silenced regions indicates a more complex regulatory mechanism, involving transitions between active and inactive states of the promoter.

1558-Pos Board B402
Feedback Control Of Fluctuations In Gene Expression And Epigenetic Memory
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Genes and proteins are organized into extensive networks that allow cells to respond and adapt to their environment. These networks are regulated through feedback loops. The dynamic behavior of a genetic network with a given architecture depends on stochastic fluctuations of the agents involved. However, it's still undetermined how the feedback strength and the noise levels couple to determine cellular behavior and its variability. In the present work, we analyze the stochastic dynamic behavior of the galactose uptake network of S. cerevisiae. We focus on how changing the strength of the positive and negative feedback loops influences circuit dynamics and adaptability, and how feedback controls