Hardly any clones arising more than six bacterial diameters behind the expanding front fixed. Closer to the front, we found the probability of fixation of P- clones to be strongly increased compared to the probability for neutral P^+ clones to reach the front. P- bacteria were expelled from the P^+ microcolony. Due to high surface tension of P^+ colonies, P- bacteria accumulated at the front of expanding colonies. This could be confirmed by characterising spontaneous mutations from the P^+ to the P- state in range expansion assays.
We conclude that the reduced physical interaction between P- and P^+ cells, leads to an increased probability of fixation for P- gonococci within a microcolony. It is tempting to speculate that gonococci can shield their major antigen, the type IV pilus, by surrounding themselves with P- cells.

1884-Pos Board B614
Dissecting the Role of Ferrous Iron in Pseudomonas Aeruginosa Gene Regulation
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In our quest towards a predictive understanding of biological systems, we have combined quantitative models of gene regulation with careful experimental tests of model predictions in order to determine how promoter architecture dictates the output level of gene expression. We have applied this quantitative framework to understand how cells sense and respond to extracellular cues, using ferrous iron-mediated gene regulation in Pseudomonas aeruginosa as an important case study.
Upon recent discovery of a two-component system that specifically responds to ferrous iron, there is an emerging picture of the critical role of Fe(II) availability in the regulatory outputs of Pseudomonas aeruginosa. On the bench top Fe(II) is readily oxidized to Fe(III), however in infections such as in the lungs of cystic fibrosis patients, cells can encounter reducing environments containing Fe(II). Ferrous iron regulates genes related to both pathogenicity and biofilm formation. These pathways are also controlled by the quorum sensing systems and the availability of ferric iron. It is not yet clear why multiple pathways are used to sense extracellular iron or how the cell integrates information from these different pathways to effectively respond to changes in the availability and oxidation state of iron.
Using statistical mechanical models of gene regulation and the tools of synthetic biology, the rules of the ferrous iron regulon are beginning to emerge. We quantified how promoter architecture dictates the regulatory response to Fe(II). A quantitative understanding of how promoters encode a ferrous iron response enabled us to predict how Fe(II) influences global gene expression patterns. Some genes respond to both Fe(II) and additional regulatory inputs, such as other transcription factors or two-component systems. We dissected these multi-input promoters to understand how cells combine ferrous iron availability with other regulatory factors to make transcriptional decisions.

1885-Pos Board B615
Using a Transcriptional Network to Approach the Mechanism of Fungal Meningitis
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C. neoformans is the most common cause of fungal meningitis, but the molecular mechanisms of pathogenesis remain poorly understood. The pivotal pathogen transcriptional regulator Gat201 inhibits phagocytosis independently of the polysaccharide capsule and plays a central role in virulence (1). To define the corresponding transcriptional network, we performed chromatin immunoprecipitation and sequencing (ChIP-Seq) and expression profiling (RNA-Seq) experiments of Gat201 and two other transcription factors, also required for virulence, regulated by Gat201 that comprise a regulatory network intimately tied to C. neoformans' virulence. Within the set of genes whose promoters are bound by all three transcription factors assayed, there are three members of a six-member protein family in C. neoformans (Blp1-6), characterized by an N-terminal signal sequence and a double-psi beta barrel motif. One member of this family, Blp1, inhibits phagocytosis through an unknown mechanism (1). In addition, we found that the promoter of a peptide required for low-density growth of a C. neoformans tup1Δ mutant strain background is also bound by all three transcription factors (2). We are currently investigating the functional roles of these secreted proteins and their biochemical mechanisms of action.
(1) Chun CD, Brown JCS, and Madhani HD. (2011) A Major Role for Capsule-Independent Phagocytosis-Inhibitory Mechanisms in Mammalian Infection by Cryptococcus neoformans. Cell Host & Microbe, 9: 243-251.
(2) Lee H, Chang YC, Nardone G, Kwon-Chung KJ. (2007) TUP1 disruption in Cryptococcus neoformans uncovers a peptide-mediated density-dependent growth phenomenon that mimics quorum sensing. Molecular Microbiology 64(3): 591-601.

1886-Pos Board B616
Altering Stochastic Noise in Gene Expression for HIV Therapy
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HIV's ability to enter a transcriptionally dormant state and establish a reservoir of latently infected cells is the major barrier to eradicating HIV from infected patients. Significant efforts are aimed at reactivating latent HIV and purging the reservoir through targeting of molecular mechanisms implicated in the establishment of latency. These efforts to perturb the latent reservoir have faced substantial challenges. Our previous studies implicated stochastic noise (i.e. fluctuations) in an HIV transcriptional positive-feedback circuit as crucial for the establishment of HIV latency and predicted that perturbing noise would alter HIV latency (Weinberger et al. Cell 2005; Dar et al. PNAS 2012). Here, we demonstrate that manipulation of stochastic noise in HIV gene expression radically perturbs HIV latency. Screening a library of small-molecule drug compounds identified over 50 compounds that modulate noise in the HIV promoter without changing the promoter's mean expression level. Strikingly, the noise-modulating compounds synergize with conventional transcriptional activators and surpass current best-in-class reactivation cocktails, while maintaining greater cell viability. Thus, noise-modulating compounds may present an approach to perturb the stability of the latent state. More generally, stochastic noise may represent a new unexplored axis for drug discovery that allows enhanced control over cell-fate specification decisions, metastasis, and pathogen persistence phenotypes.

1887-Pos Board B617
Correlating Rat Basophil Leukemia Cell Activation with Interleukin 4 RNA Production using Single Molecule Fluorescence In-Situ Hybridization, Automated Super-Resolution Microscopy, and GPU-Enabled Image Analysis
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Single-molecule, single-cell studies of genetic expression have provided key insights into how cells respond to external stimuli. Direct single-cell measurements of individual bio-molecules, such as small RNA (sRNA) or messenger RNA (mRNA) transcripts, make it possible to quantify the heterogeneity in spatiotemporal responses of key signaling and regulatory processes. Critically, these investigations provide information on cellular fluctuations-information that is hidden by typical biochemical ensemble measurement approaches. Recent work has suggested that macroscopic cell properties, such as cell morphology, are correlated with gene expression. Here we present single-cell studies of a signal-activated gene network: Interleukin 4 (IL4) RNA production in rat basophil leukemia (RBL) cells during the allergic response. IL4 mRNA production has been closely linked to histamine release by RBL cells in ensemble measurements. To further investigate this regulatory network, we fluorescently label individual IL4 RNA transcripts in populations of RBL cells, subject to varying external stimuli. A custom super-resolution microscope and GPU-accelerated data analysis package are used to measure the number of fluorescently labeled IL4 transcripts in populations of RBL cells on a cell-by-cell basis. To test the hypothesis that RBL cell morphology may connected to IL4 production and histamine release, we analyze white light images of RBL cells and cross-reference cell morphology with IL4 RNA levels. We find that the activation of RBL cells, determined by white-light imaging, is well correlated with IL4 mRNA expression yet highly heterogeneous for certain stimuli.

1888-Pos Board B618
Extrinsic Noise Driven Phenotype Switching in a Self-Regulating Gene
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Analysis of complex gene regulation networks gives rise to a landscape of metastable phenotypic states for cells. Heterogeneity within a population arises due to infrequent noise-driven transitions of individual cells between nearby metastable states. While most previous works have focused on the role of intrinsic fluctuations in driving such transitions, in this work we investigate the role of extrinsic fluctuations. First, we develop an analytical framework to study the combined effect of intrinsic and extrinsic noise on a toy model of a Boolean regulated genetic switch. We then extend these ideas to a more biologically relevant model with a Hill-like regulatory function. Employing our theory and extensive Monte Carlo simulations, we show that extrinsic noise

can significantly alter the lifetimes of the phenotypic states and may fundamentally change the escape mechanism. Finally, we show that our theory can be generalized to more complex decision making networks in biology.

1889-Pos Board B619
Imaging Chromosome Structure in Bacteria by Super-Resolution Microscopy
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Fluorescence microscopy is a powerful quantitative tool for exploring regulatory networks in single cells. However, the number of molecular species that can be measured simultaneously is limited by the spectral separability of fluorophores. Here we demonstrate a simple but general strategy to drastically increase the capacity for multiplex detection of molecules in single cells by using optical super-resolution microscopy (SRM) and combinatorial labeling. The basis for this new approach are the following: given the 10 nanometers resolution of a super-resolution microscope and a typical cell a size of (10um)3, individual cells contains effectively 109 super-resolution pixels or bits of information. Most eukaryotic cells have 104 genes and cellular abundances of 10-100 copies per transcript. Thus, under a super-resolution microscope, an individual cell has 1000 times more pixel volume or information capacities than is needed to encode all transcripts within that cell. As a proof of principle, we labeled mRNAs with unique combinations of fluorophores using Fluorescence in situ Hybridization (FISH), and resolved the sequences and combinations of fluorophores with SRM. We measured the mRNA levels of 32 genes simultaneously in single cells. In addition, we have performed DNA-FISH experiments simultaneously with RNA-FISH to image both the chromosome structure and transcription in single E.coli cells.

1890-Pos Board B620
Stochastic Fluctuations Link Promoter Chromatin Structure and Gene Expression
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Gene expression noise is characterized by random fluctuations in the number of mRNA and protein molecules produced from single genes throughout a cell population. The underlying molecular basis for this random on/off switching of genes was unknown, but hypothesized to arise from stochastic fluctuations in promoter chromatin structure. To address this question, we isolated single PHO5 gene molecules from yeast and mapped their steady-state promoter nucleosome configurations by electron microscopy. We found that the activated PHO5 promoter adopted eight distinct nucleosome configurations including the fully nucleosomal and nucleosome-free states. The probability of observing each of these eight configurations was explained by a stochastic process of nucleosome assembly, disassembly, and sliding and was tuned by the strength of Pho4, the transcriptional activator for PHO5. Downstream PHO5 expression was strongly influenced by promoter nucleosome fluctuations, mechanistically linking gene expression noise to promoter nucleosome dynamics. Further analysis has focused on chromatin remodelers and the role they play in generating promoter nucleosome probability distributions. Our findings suggest that stochastic promoter chromatin remodeling is the basis for transcriptional bursting and that activators, and the remodeling proteins they recruit, play a critical role in generating distinct promoter nucleosome probability distributions.

1891-Pos Board B621
Structure and Function of a Transcriptional 'Accelerator' Circuit
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Biological signaling circuits, like electrical circuits, face a fundamental tradeoff between speed and amplitude: faster rates of initial increase are typically obtained at the cost of a higher steady-state level. This creates an evolutionary tradeoff when rapid signaling is essential but the signaling molecule is cytotoxic at high levels (e.g. for fever response, inflammatory cytokines, and many viruses). We recently discovered a transcriptional circuit in a human herpesvirus (CMV) that overcomes this tradeoff - and confers significant fitness to the virus - by converting signaling inputs into faster expression rates without amplifying final equilibrium levels in individual cells (Teng et al. Cell, 2012). Strikingly, the accelerator circuit maps to a transcriptional negative-feedback loop encoding an exceptionally high self cooperativity (Hill coefficient $\approx$ 7). Binding of the virus's essential transactivator protein, IE2, to a single 14-bp sequence in its own promoter generates negative auto-regulation but how such a high Hill coefficient was generated remained unclear. Here, we report biophysical and structural studies of the IE2-DNA interaction showing a novel homo-multimer structure accounts for Hill coefficient ~ 7. In general, such accelerator circuits may provide a mechanism for signal-transduction circuits to respond quickly to external signals without increasing steady-state levels of potentially cytotoxic molecules.

1892-Pos Board B622
Deciphering Transcriptional Dynamics in Vivo by Counting Nascent RNAs
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Transcription of a gene by an RNA polymerase is the focus of most forms of regulation of gene expression. Even though careful biochemical experimentation has revealed the molecular mechanisms of transcriptional regulation for a number of different promoters, the question of how transcription is regulated in cells is still poorly understood. Recently experimental methods have been developed for counting the number of nascent RNA molecules in a single cell for a gene of interest. We show theoretically that measurements of the cell-to-cell variability in the number of nascent RNAs can be used to test different mechanisms of transcription initiation. In particular, we derive exact expressions for the first two moments of the distribution of nascent RNA molecules for an arbitrary mechanism of transcription initiation. These theoretical results are applied to published data on the nascent RNA distributions obtained for a collection of constitutively expressed yeast genes. We find that the data is not consistent with a single-step mechanism of initiation, which has been generally assumed. Instead, a two-step mechanism of initiation with both steps of equal duration is consistent with the available data. These findings for the yeast promoters highlight the utility of our theory for deciphering transcriptional dynamics in vivo.

1893-Pos Board B623
Exploring Feedback Regulation in the S. Cerevisiae PKA Pathway
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Protein kinase A (PKA) in S. cerevisiae is a central mediator of environmental nutrient sensing that modulates cellular growth and stress responses based on environmental conditions. In mammalian cells, PKA plays an equally important function in regulating growth, development, and metabolism. PKA activity is hence intricately regulated by multiple feedback loops. There are at least two known negative feedback loops regulating the synthesis and degradation of cAMP, the small molecule activator of PKA. While the individual components of the PKA regulatory network are known, we still lack understanding of the contribution of the different feedback loops to regulation in this system. Equally missing is a quantitative characterization of the loops that endow the system with its distinct dynamical features. In this work, we use a precise optogenetic input to study the dynamical properties and feedback regulation of the PKA network . Using time-lapse fluorescence microscopy, we measure the quantitative dynamics of PKA signaling in response to different light pulses for cells with an intact PKA network, in addition to cells where different feedback loops have been perturbed. We quantify the relative essentialness, strengths, and timescales of the feedback loops and organize these data in a computational model that provides predictive understanding of how changes in the feedback parameters tune the dynamical profile of the system.

1894-Pos Board B624
Ribosome Profiling of the Caulobacter Cell-Cycle
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An important goal in understanding cellular differentiation is to determine how the genetic information encoded in the genome is expressed properly in time and space to yield cell types with distinct functions. The bacterium *Caulobacter crescentus* has proven to be a valuable model organism for studying cellular differentiation processes that occur as a function of the cell cycle. Each *Caulobacter* cell division is asymmetric, yielding daughter cells with different cell fates. This process requires rapid and specific changes in gene expression during the cell cycle that are controlled at many levels, including transcriptional regulation, transient DNA methylation, differential proteolysis, and protein phospho-signaling. However, relatively little is known about the cell cycle control of mRNA translation. To understand the role of translational regulation in *Caulobacter* differentiation and asymmetric cell division, we are employing ribosome profiling to monitor genome-wide changes in translation at multiple times during cell cycle progression. Arrested ribosomes are treated with ribonuclease to partially digest the mRNA, yielding short fragments protected by