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