2712-Pos Board B142
Live-Cell Single-Molecule Imaging of Sense and Antisense Transcription of a Yeast Gene
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The eukaryotic genome is pervasively transcribed, giving rise to various sorts of non-coding RNAs whose mechanisms of action are for the most part not understood. Recent technological advances now allow direct visualization of the synthesis of nascent transcripts from individual genes over time by decorating RNAs with fluorescent proteins. Using the orthogonal RNA-binding MS2 and PP7 bacteriophage coat proteins, we were recently able to tag two regions of the same RNA in two different colors [Coulon et al. 2014, eLife, in press]. Here, we used this technique to visualize simultaneously sense and antisense transcription from the GAL10 locus in yeast, during activation of the GAL pathway. Fluorescence fluctuations recorded in both channels at the transcription site reflect the kinetics of transcription on both strands as the GAL10 gene gets activated in response to galactose. We observe transient antisense transcription occurring almost exclusively prior to the appearance of sense transcription. Using cross-correlation analysis, we uncovered specific temporal windows relatively to sense activation where antisense transcription is enriched or depleted - likely reflecting the biochemical mechanisms underlying activation. Once transcription of the GAL10 gene starts, transcripts are produced in bursts separated by periods of inactivity, occasionally leaving the opportunity for antisense transcription to happen. We developed a method for applying fluctuation correlation analysis to non-stationary time traces. This allowed us to isolate the bursting kinetics even in the non-steadystate context of a transient response to galactose. By modeling the autocorrelation of a bursting gene, we were able to infer from our data how the elongation rate, burst size and burst frequency of the GAL10 gene are modulated by different doses of galactose. This work shows how in vivo single-molecule methods and fluctuation analysis can reveal unanticipated mechanisms of transcriptional regulation.

2713-Pos Board B143
The Mechanism of Transcription Stalling under Torsion
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During transcription, RNA polymerase (RNAP) translocates along DNA and introduces torsional stress, and excessive torque accumulation can lead to transcription stalling. We previously measured this stall torque for *E. coli* RNAP during transcription[1]. However the mechanism and regulation of RNAP stalling under torsion has remained elusive. Here, we investigate the transcriptional dynamics of RNAP in the presence and absence of GreB, a transcription elongation factor known to rescue backtracked RNAP. Using an angular optical trap assay, we found that the presence of GreB can significantly increase the stall torque of RNAP. In addition, RNAP often exhibited distinct reverse motion upon stalling in the absence of GreB, while this was rarely observed in the presence of GreB. These results suggest that backtracking is the primary mechanism of RNAP stalling on DNA under torsion and demonstrate a potential regulatory role for GreB in assisting an elongating RNAP in overcoming the torsional barrier of DNA.
[1]J. Ma, L Bai, and M.D. Wang. Transcription under torsion. Science 340:1580-3 (2013).

2714-Pos Board B144
A High-Throughput Single-Molecule Assay for Screening Transcriptional Interference
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Transcription of genetic information from DNA to RNA for protein synthesis and regulatory control is a fundamental biological process. Gene expression in the cell is regulated, and eventually silenced, for various purposes such as to trigger a developmental pathway, to protect the genome from infectious DNA elements or to respond to environmental stimuli. Gene silencing techniques are used in biomedical research and in the development of novel therapeutics for treating various cancers, infectious diseases and neurodegenerative disorders.
We present a simple single-molecule assay for studying transcription and assessing the effects of small molecules on gene transcription. Our approach combines aspects of tethered particle motion (TPM) microscopy with total internal reflection fluorescence (TIRF) microscopy. This method allows us to watch RNA transcription occurring on dozens of single DNA molecules in parallel and may be scaled for use in higher-throughput investigations of transcriptional activity.
To illustrate the utility of our method, we have been investigating transcriptional interference caused by the histone-like nucleosomal protein (H-NS), which is a global transcriptional silencer abundant in many bacteria. Here we use this technique to explore the promoter occupancy and procession of T7 polymerase in the presence of H-NS. However, our setup is amenable to the study of a host of different regulatory factors acting on a variety of promoter architectures.

2715-Pos Board B145
DNA Looping both Enhances and Suppreses Transcriptional Noise
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DNA looping has been observed to enhance and suppress transcriptional noise but it is uncertain which of these two opposite effects is to be expected for given conditions. Here, we present the derivation of analytical expressions for the main quantifiers of transcriptional noise in terms of the molecular parameters and elucidate the role of DNA looping [1]. Our results rationalize paradoxical experimental observations and provide the first quantitative explanation of landmark individual-cell measurements at the single molecule level on the classical lac operon genetic system [2].
[1] Vilar J.M.G. and Saiz L., Physical Review E 89, (6) 062703 (2014).
[2] Choi et al., Science 322, 442-446 (2008).

2716-Pos Board B146
Nuclear Actin Dynamics Regulate Nuclear Organization and Transcription
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Actin is an important and elegant mechanotransducer that transfers messages from the extracellular environment into the nucleus, thus conferring changes in both the physical properties and the genetic program of the cell. Although actin in the nucleus has been established in a growing number of functions, the form of nuclear actin remains poorly understood as there are no apparent actin filaments in the nucleus. Therefore, we tested how disrupting the form of nuclear actin impacts function and what effects this may have on the nucleus. To do so, we formed stable nuclear actin filaments using a variety of methods, including the nuclear enrichment of actin binding proteins supervillin and α-E-catenin and the expression of a mutant form of skeletal α-actin. Using fluorescence recovery after photobleaching (FRAP), we found that stabilization of nuclear actin filaments significantly impairs actin dynamics within the nucleus. The formation of nuclear actin filaments coincides with striking changes in nuclear structure and overall nuclear topography as determined by confocal microscopy and raster image correlation spectroscopy. Using a combination of FRAP analysis, structured illumination microscopy and immunological assays, we found that nuclear actin filaments reduce the association of actin with RNA polymerase II and this correlates with impaired RNA polymerase II dynamics, localization and gene recruitment. Moreover, we were able to recapitulate our findings in purified nuclear extract by using in vitro transcription assays with the covalent actin crosslinking domain (ACD) of V. cholerae MARTX toxin. Based on our data, which help explain the absence of nuclear actin filaments in the interphase mammalian nucleus, we propose a model where nuclear actin dynamics are critical for maintaining proper nuclear function.

Chromatin and the Nucleoid

2717-Pos Board B147
Epigenetics: How Much Physics Do We Need to Understand It?
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Eukaryotic genomes are divided into chromosomes, each consisting of a single molecule of several centimeters of DNA compacted into a nucleoprotein