Platform: Transcription

1150-Plat
Quantifying the Interaction Between Neighboring Gene Circuits
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In heterologous gene expression, a module with two gene circuits responsible for expression of a foreign gene of interest and selection is integrated into the host cell genome. Although the expression level of the foreign gene is generally understood to be determined by the strength of the promoter immediately upstream, several studies have shown that it varies significantly depending on the site and orientation of genomic integration. In addition, the arrangement of the selection circuit within the module may also affect the gene expression level. To test this possibility, we built a set of heterologous modules where two different gene circuits are placed right next to each other in all possible relative orientations. The activities of these gene circuits can be separately driven by external inducers and simultaneously monitored from fluorescent protein levels. We integrated these modules into the genome of budding yeast and quantitatively measured expression levels of both gene circuits at multiple inducer-level combinations. Our preliminary results show that the adjacent gene circuits interact in a cooperative and reciprocal manner; activation of the first gene circuit enhances the expression level of the second gene circuit and vice versa. Moreover, the degree of enhancement depends on the relative orientation of the gene circuits, highest when the promoters are pointing away from each other. Based on our results, we present a rate-equation based model to highlight potential mechanisms of promoter interaction.

1151-Plat
Visualizing the Transcription Cycle of Endogenous RNA Polymerase II in Single Living Cells
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How efficient is transcription in vivo? Recent fluorescence microscopy studies have begun to confront this question by measuring the binding times of RNA polymerase II (pol II) and other components of the transcription machinery in single living cells. This has revealed an extremely dynamic transcription machine that appears to operate with components in continual flux. However, it remains debatable whether or not these rapid dynamics lead to efficient transcription, mainly because it has been difficult to distinguish different phases of the transcription cycle in vivo. For example, using GFP it is possible to visualize the movement of pol II, but directly distinguishing the unmitted fraction from the initiated or elongating fractions has not yet been possible. To overcome this difficulty, we have loaded fluorescent antibody fragments (Fab) against unphosphorylated and phosphorylated forms of pol II into living cells containing an inducible tandem gene array. Using this unique system, we are quantifying the accumulation of uninitiated (unphosphorylated), initiated (ser 5 phosphorylated), and elongating (ser 2 phosphorylated) forms of pol II to the gene array after induction. Our observations suggest transcription is quite efficient, with pol II being recruited to activated genes within ~3 minutes time, after which ~70% are initiated within 30 seconds and ~35% proceed to elongation within another 30 seconds. We are currently investigating how these transcription dynamics correlate with histone modification dynamics at the gene array.

1152-Plat
Experimental and Stochastic Model Analysis of the Influence of SIC1, CLN2 and CLB5 Transcriptional Noise on the Timing Regulation of G1/S Transition in S. Cerevisiae Cell-Cycle
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Gene expression results in significant fluctuations in transcriptional and translational abundance among cells of a genetically identical population. Remarkably, strong regulatory mechanisms in Saccharomyces cerevisiae cell-cycle enable its precision despite these fluctuations. Our work combining modeling and experimental approaches investigates the influence of stochasticity in gene expression, especially in transcription, for timing regulation of the G1/S transition. We focus on SIC1, a cyclin-dependent kinase inhibitor, and its interacting partners, the cyclins Cln2 and Clb5. The interplay of these cell-cycle regulators is critical for precise timing of the G1-phase exit. We quantify SIC1 transcripts level and transcriptional noise in a cell population via fluorescence microscopy by taking advantage of two highly sensitive single molecule detection methods, the MS2-CP and the Fluorescence in situ Hybridisation (FISH) techniques. We used these data to explore a stochastic model for the G1/S transition timing regulation focusing on the fluctuations of SIC1 transcripts level.

The experimental investigation revealed a distribution between 0 and 10 SIC1 mRNAs per cell and a significant increase of transcripts in the G1-phase. Our model predicts that by producing only a few SIC1 mRNAs, as observed in our experiments, the cell ensures low noise level of S1C1 protein and an exact timing of the S-phase entrance.

We conclude that timing properties in yeast cell-cycle are highly influenced on the molecular level by discrete abundance of transcription products. Essentially, transcription regulation is involved in maintaining robustness in cell-cycle progression. To obtain a comprehensive understanding of transcription regulation at an interacting-proteins-network level, we are now measuring CLN2 and CLB5 transcript levels to investigate the correlation of their transcript levels as well as that of SIC1.

1153-Plat
Single Molecule Probing of the Human Tumor Suppression Transcription Initiation Machinery
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In humans, transcription Pre-Initiation Complex (PIC) assembly begins with the recognition of the core promoter by the large multi-subunit TFIIID complex and culminates with recruitment of RNA Polymerase II. Transcriptional activators, such as the tumor suppressor p53 protein, stimulate transcription initiation, in part by binding sequences upstream of the promoter and help recruit TFIIID to core promoter DNA. In response to cellular stress the tumor suppressor p53 protein becomes activated and turns on a variety of target genes in a highly coordinated manner to dictate cellular fate. Cellular levels of p53 are essential in dictating gene expression programs based upon the affinity of p53 for binding sites upstream of the core promoter. Many general transcription factors that bind to the core promoter are known to interact with p53 and potentially alter the affinity of each factor for the promoter DNA. Despite almost 20 years of research, the dynamic interplay between p53 and components within the general transcription machinery at physiological promoters remains poorly understood.

We have probed in real-time the association between human p53, TFIIID, and various p53 target gene promoters, using a combination of in vitro single molecule tethered particle motion (TPM) and high-resolution TIRF co-localization assays. Our results show that p53 and TFIIID act cooperatively to aid in recruitment to promoter DNA. In addition, differences in cooperative assembly of p53 and TFIIID on various physiological promoters underscore a mechanism for p53’s graded response to cellular stress. TPM analysis reveals p53 and TFIIID dependent DNA looping suggests stable interactions between upstream bound activators and core promoter bound factors. We have further examined the influence of additional general transcription factors on p53 mediated transcription complex assembly.

1154-Plat
Single-Molecule Insights on the Human RNA Polymerase II Transcription Regulation: Assembly of the Pre-Initiation Complex, Re-Initiation Scaffold, and the Roles of Activators
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Transcription of all protein-coding genes in human cells begins with the assembly of the Pre-Initiation Complex, Re-Initiation Scaffold, and the Roles of Activators. Molecular Biophysics, Humboldt University of Berlin, Berlin, Germany.
Gene expression results in significant fluctuations in transcriptional and translational abundance among cells of a genetically identical population. Remarkably, strong regulatory mechanisms in Saccharomyces cerevisiae cell-cycle enable its precision despite these fluctuations. Our work combining modeling and experimental approaches investigates the influence of stochasticity in gene expression, especially in transcription, for timing regulation of the G1/S transition. We focus on SIC1, a cyclin-dependent kinase inhibitor, and its interacting partners, the cyclins Cln2 and Clb5. The interplay of these cell-cycle regulators is critical for precise timing of the G1-phase exit. We quantify SIC1 transcripts level and transcriptional noise in a cell population via fluorescence microscopy by taking advantage of two highly sensitive single molecule detection methods, the MS2-CP and the Fluorescence in situ Hybridisation (FISH) techniques. We used these data to explore a stochastic model for the G1/S transition timing regulation focusing on the fluctuations of SIC1 transcripts level.

The experimental investigation revealed a distribution between 0 and 10 SIC1 mRNAs per cell and a significant increase of transcripts in the G1-phase. Our model predicts that by producing only a few SIC1 mRNAs, as observed in our experiments, the cell ensures low noise level of S1C1 protein and an exact timing of the S-phase entrance.

We conclude that timing properties in yeast cell-cycle are highly influenced on the molecular level by discrete abundance of transcription products. Essentially, transcription regulation is involved in maintaining robustness in cell-cycle progression. To obtain a comprehensive understanding of transcription regulation at an interacting-proteins-network level, we are now measuring CLN2 and CLB5 transcript levels to investigate the correlation of their transcript levels as well as that of SIC1.