Transcription

1146-Pos Board B123

Pausing in *Escherichia coli* Transcription Initiation

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An essential and highly regulated step in gene expression is transcription initiation. After promoter binding and DNA unwinding ('bubble opening') and in the presence of nucleoside triphosphates (NTPs), the RNA polymerase (RNAP)-promoter initial transcribing complex (RPitc) engages in 'abortive initiation', a process in which RNAP cycles between synthesis and release of short RNA transcripts. In abortive initiation, RPitc is believed to undergo a sequence of transitions between different initiation sub-states. The kinetics of the production of a full RNA transcript starting at a late initiation sub-state is expected to be similar or faster than the kinetics measured from an earlier initiation sub-state. To test this hypothesis, we developed a novel *in vitro* single-run quenched kinetics transcription assay based on the detection and quantification of run-off transcripts. Using this assay and corroborating it with gel-based and magnetic tweezer assays, testing two different promoters, we surprisingly found that run-off transcription kinetics starting from late initiation sub-states is slower than kinetics starting from earlier initiation sub-states. When the same kinetic measurements were performed in the presence of the transcription elongation factor GreA, the kinetics starting from a late initiation sub-state was accelerated. Experimental results suggest that as a function of shortage in NTPs RPitc can enter an off-pathway state in which the nascent RNA is in a backtracked, paused position, awaiting incorporation of the missing NTP. Our findings suggest that pausing at distinct stages of transcription initiation could regulate gene expression under stressed conditions.

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Walk and Check along a Viral RNA Polymerase Transcription Elongation Path

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Gene transcription is a fundamental step in the central dogma of molecular biology. The essential protein enzymes that direct the process are RNA polymerases (RNAPs). The viral RNAP from bacteriophage T7 serves a self-sufficient transcription engine and is widely used for lab gene expression and engineering, although its operational mechanisms remain controversial. We have systematically studied mechano-chemical coupling and fidelity control mechanisms of T7 RNAP during its elongation. Based on single molecule measurements and high-resolution structures, we had demonstrated that a small translocation free energy bias aids nucleotide selection in T7 RNAP [1]. Accordingly, we performed atomistic molecular dynamics (MD) simulation and discovered how a critical tyrosine residue aids the nucleotide selection for selective Brownian ratcheting of the RNAP [2]. In addition, we also built a theoretical framework to analyze efficient stepwise nucleotide selection of the RNAP in the absence of proofreading [3]. Recently, we constructed the Markov state model for the PPi product release and translocation of T7 RNAP implementing extensive MD simulations. In particular, we noticed a universal module in which an essential lysine/arginine residue greatly assists a jump-from-cavity activation process of the PPi release [4]. The activated PPi release is unlikely to drive the RNAP translocation, thus ruling out a power stroke mechanism during the RNAP elongation. Overall, our work demonstrates how physical modeling and large-scale simulations can be combined to reveal underlying mechanisms of a key physiological process that may not be easily accessible from experimental approaches.

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1148-Pos Board B125

Transcription Factor Clustering in Live Yeast Cells

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Genes are regulated by a family of proteins called transcription factors which operate by binding to specific sequences of DNA. These sequences are short and occur frequently in the genome and it is unclear how transcription factors find their correct sites. We have investigated two zinc-finger transcription factors, Mig11 and Msn2, which are both glucose dependant repressors of genes. These proteins have been labelled with the green fluorescent protein (GFP) by chromosomal integration and another protein Nrd1 has been labelled with the mcherry fluorescent protein as a marker for the nucleus. Using single-molecule fluorescence microscopy2 in different extra-cellular glucose conditions, we are able to observe individual molecules of these proteins at different stages: bound to DNA in the nucleus, translocating across the nuclear membrane and diffusing in the cytoplasm. We show that these proteins operate in clusters through all these stages, in the nucleus and cytoplasm. Forming clusters may facilitate transcription factors finding their correct sites3 on the genome and provide possible evidence for transcription factories.

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1149-Pos Board B126

E.Coli RNA Polymerase Activity under Crowding

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Biological reactions in the cellular environment differ physicochemically from those performed in dilute buffer solutions due to the high viscosity and crowding effects associated with the high density of macromolecules in the cell. Earlier work has shown that in vivo bacterial transcription is affected by the cellular environment, involving slower diffusion of various components in the cellular milieu (due to high viscosity), increase in their local concentrations, and modulation of their binding affinities. However, since most studies are focused on multiple cycles of either transcription or association of DNA and RNA Polymerase (RNAP) to make RNAP open complex (RPo), the effect of the crowded environment on a single transcription run is still poorly understood. Here, we have developed and applied a novel transcription quenched-kinetics assay using single-molecule detection to investigate the size/concentration effects of various osmolytes and macromolecular crowding agents, which mimic the crowded cellular environment, on actively-transcribing RNAP. Our results demonstrate an expected slowdown of transcription kinetics due to increased viscosity, and unexpected enhancement in transcription activity by larger crowding agents at the same viscosity. These findings suggest that crowding agents affect the transcription reaction after RPo formation through volume exclusion.

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Live Cell Single Molecule Binding of Transcription Factors in Living Cells. Characterizing p53 Latency

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The binding of transcription factors (TFs) to their regulatory sites on DNA determines how much and how timely a particular gene will be expressed, and ultimately how the cell respond to external cues. TF binding is typically studied by bulk biochemical experiments as chromatin immunoprecipitation (ChIP) As these methods provide limited temporal resolution and they are unable to provide information about these interactions at the single cell level, the

interpretation of ChIP results can be challenging when dealing with TFs exhibiting rapid turnover, or with cells and tissues exhibiting a patterned non-homogeneous transcriptional response to an external stimulus.
Here we describe a microscopy-based single molecule imaging approach which can be used to obtain direct information on the TF binding kinetics to chromatin with the sub-second temporal resolution at the individual live-cell level [1,2]. We apply this method to characterize the binding of the tumor suppressor p53 both in basal, non-stimulated conditions and upon its activation by genotoxic stress induced by ionizing radiation: we show that p53 binds transiently to DNA (timescale of seconds), and that this interaction is modulated following the induction of damage, Importantly, more stable interactions are associated to higher transcription rates of p53 target genes, indicating that p53 acts as a latent TF, reviving an hypothesis initially derived from in-vitro studies [3], but later challenged by low temporal resolution ChIP experiments [4].
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1151-Pos Board B128
Nanoscale Probing of the p53 Tumor Suppression Transcription Machinery
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More than 50% of cancer patients harbor p53 mutations, highlighting the essential role of p53 in tumor suppression. In response to various stress signals, p53 targets the TFIID-mediated transcription machinery to stimulate expression of vast gene networks involved in diverse cellular pathways. Understanding how p53 turns on TFIID-mediated transcription initiation at the single molecule level will advance our knowledge of p53's tumor suppression activity.
Despite 20 years of biochemical studies, it remains elusive how p53 recruits TFIID and other basal transcription factors (e.g. TFIIB and RNA Polymerase II) to facilitate pre-initiation complex formation on various target gene promoters. We hypothesize that p53 induces distinct DNA-binding conformations within basal factors to stimulate assembly culminated in transcription initiation. Therefore, to test our hypothesis, we employed an innovative integrated approach involving single particle cryo-electron microscopy (EM), single molecule fluoresce microscopy and biochemistry.
Intriguingly, our studies demonstrated that p53 delivers and promotes stable binding of TFIID to DNA. Unexpectedly, the interaction of TFIID and DNA in turn causes p53 to quickly dissociate from the assembly and promoter. Furthermore, our work suggest that the direct association of p53 and TFIID is sufficient to recruit TFIID onto various target gene promoters. More significantly, p53 induces a common DNA-binding conformation of TFIID. We further identified a novel role of TFIID in anchoring core promoter DNA downstream of the transcription start site.
Collectively, these findings indicate that p53, and potentially activators in general, serve as escorts to dynamically recruit and load the basal transcription machinery onto DNA. Importantly, p53 facilitates formation of common DNA-binding forms of basal factors. Overall, our studies are critical for understanding how eukaryotic transcription complexes initiate transcription on different types of protein-coding genes in response to various stress signals.

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Rapid Long Range Sliding of RNA Dependent RNA Polymerases on Viral Genome Templates Visualized by Photoactivatable Localization Microscopy
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While DNA binding proteins like the lac repressor have been observed to slide efficiently on the DNA template, the eukaryotic DNA dependent RNA polymerases were shown to bind their promoters directly from solution. Initiation of transcription remains less understood for negative sense RNA viruses which include major human and animal pathogens such as Ebola, measles, Rabies, VSV and Influenza virus. The viral genomic RNA is encapsidated in thousands of copies of nucleoprotein N (~30kD), which can efficiently shield the full stretch of RNA genome and protect the RNA to be inaccessible even to the RNAses. To transcribe this genomic RNA, viruses package tens of copies of a specialized RNA dependent RNA polymerase which is composed of a catalytic subunit L (~250kD) and an N-RNA binding protein p (~30kD). While the full genome N-RNA can be longer than 4 microns, transcription can only initiate from the 3' end. We have previously measured the binding affinity of the RdRPs to the N-RNA genome template and have shown that RdRPs have a dissociation constant of less that 20pM. Theoretical predictions of feasible transcription models all have pointed out to the essential requirement that the RdRPs slide on the N-RNA genome templates. Here we have created a recombinant VSV virus that has L protein fused with the Dendra2 fluorescent protein. We report tracking of single RdRPs along genome templates using photoactivatable localization microscopy. Our observations show rapid sliding of single RdRPs with diffusion coefficients as high as 106 nm^2/sec and distances as long as 800 nanometers. These observations confirm theoretical studies predicting one-dimensional diffusion for RdRPs on VSV's genome templates, and also unmask a fundamental mechanism of redistribution of polymerases on the genome templates.

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PPi Release Followed by DNA Translocation Studied from Atomistic Simulations of T7 RNA Polymerase Transcription
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Bacteriophage T7 RNA polymerase (RNAP) serves a prototypical system to study general physical mechanisms of transcription due to its relative simple structures along with self-sufficient and strong transcription activities. We computationally study the pyrophosphate ion (PPi) product release and DNA translocation processes during the T7 RNAP elongation with unprecedented structural dynamics detail. We implemented extensive all-atom molecular dynamics (MD) simulations and constructed the Markov state model (MSM). The MSM reveals a jump-from-cavity PPi release mechanism, which distinguishes from charge facilitated hopping mechanisms identified previously for bacterial and eukaryotic multi-subunit RNAPs. Furthermore, we performed steered MD simulations and microsecond long MD simulations to explore additional couplings and slow motions. We found that the PPi release does not appear to be tightly coupled to opening of an O-helix that is directly tied to the DNA translocation. Hence, the study disfavors a power stroke mechanism of the RNAP elongation. In particular, we discovered a key lysine/arginine residue that assists the PPi release and the module appears to be universal not only to structurally similar polymerases but also to the multi-subunit RNAPs, even though their overall structures and mechanisms look quite different. We also want to understand why the viral RNAP does not backtrack during its Brownian ratcheting.

1154-Pos Board B131
Regulation of the BDNF mRNA 3' UTR on the RNA Level
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Brain-derived neurotrophic factor (BDNF) is part of the neurotrophic family of genes that encodes for proteins that are known to promote survival of neurons in the peripheral and central nervous systems. The expression of the BDNF gene results in the production of two mRNAs with different lengths, one with a short 3' untranslated region (UTR) and the second with a long 3'-UTR. The long 3'-UTR BDNF mRNA is the only transcript found in the dendrites and sequences unique to this transcript could be implicated in the regulation of local protein synthesis at distal sites. In this study we used biophysical methods such as circular dichroism spectroscopy, UV/Vis spectroscopy, nuclear magnetic resonance spectroscopy as well as steady-state fluorescence spectroscopy to investigate the potential of a 26 nucleotide RNA sequence located in the BDNF mRNA long 3'-UTR to adopt a G quadruplex structure and analyzed its interactions with the fragile X mental retardation protein, a translation regulator of dendritic mRNAs.

1155-Pos Board B132
Subnuclear Spatial Structuring of Chromatin and Polymerase II during Transcription Activation of the Zebrafish Zygotic Genome
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The relevance of spatial organization of chromatin to regulation of transcription is increasingly recognized [1]. Conversely, it was recently suggested that the level of general transcription activity affects subnuclear chromatin distribution [2]. Here, we ask how a general increase in transcription by polymerase II changes subnuclear chromatin distribution. Specifically, we used the transition of early zebrafish embryos from transcription quiescence to fully established