and essentially stops transcription initiation. Inhibited transcription can be recovered upon gyrase binding and reaction on the DNA. Furthermore, using single-cell mRNA counting fluorescence in situ hybridization (FISH) assay, we find the extent of transcriptional bursting depends on the intracellular gyrase concentration. These findings prove that transcriptional bursting of highly expressed genes in bacteria is primarily caused by reversible switching between different chromosomal supercoiling levels.

2451-Pos Board B143
Watching the RNA Polymerase Transcription by Time-Dependent Soak-Trigger-Freeze X-Ray Crystallography
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The challenge for structural biology is to understand atomic-level macromolecular motions during enzymatic reaction. X-ray crystallography can reveal high resolution structures; however, one perceived limitation is that it reveals only static views. Here we use time-dependent soak-trigger-freeze X-ray crystallography, namely, soaking nucleotide and divalent metal into the bacteriophage RNA polymerase (RNAP)-promoter DNA complex crystals to trigger the nucleotidyl transfer reaction and freezing crystals at different time points, to capture real-time intermediates in the pathway of transcription. In each crystal structure, different intensities and shapes of electron density maps corresponding to the nucleotide and metal were revealed at the RNAP active site which allow watching the nucleotide and metal bindings and the phosphodiester bond formation in a time perspective. Our study provides the temporal order of substrate assembly and metal co-factor binding at the active site of enzyme which completes our understanding of the two-metal-ion mechanism and fidelity mechanism in single-subunit RNAPs. The nucleotide-binding metal (MeB) is coordinated at the active site prior to the catalytic metal (MeA). MeA coordination is only temporal, established just before and dissociated immediately after phosphodiester bond formation. We captured these elusive intermediates exploiting the slow enzymatic reaction in crystallo. These results demonstrate that the simple time-dependent soak-trigger-freeze X-ray crystallography offers a direct means for monitoring enzymatic reactions. [Ref: Basu, R.S., and K.S. Murakami (2013). J Biol Chem., 288, 3305-3311]

2452-Pos Board B144
Structural and Dynamic Regulation of TFIID-Mediated Transcription Initiation Complex Assembly by the Tumor Suppressor P53 Protein
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p53 plays a central role in tumor suppression. To quickly respond to diverse stress stimuli, p53 binds specific elements in various target promoters to induce vast gene networks for maintaining cellular integrity. p53 stimulates transcription in part by aiding promoter recruitment of the transcription machinery. TFIID, a key component within the transcription machinery, is responsible for binding specific core promoter DNA sequences and recruiting other basal factors including RNA Polymerase II to initiate transcription. However, the exact mechanism underlying how p53 facilitates TFIID-mediated transcription is unclear.
Each p53 target gene has a unique arrangement of p53-responsive and core promoter elements. How these various arrangements on different gene promoters regulate the structural architecture of TFIID and the positioning of p53/specific elements remains unknown. Moreover, structural information of p53 bound to its various target promoters and other factors remains elusive. Therefore, we aim to decipher the molecular mechanism underlying p53's ability to stimulate transcription by revealing the biochemical, structural and dynamic basis of TFIID bound to p53 and promoter DNA.
To this end, we established unique protein purification strategies to generate high-purity native TFIID complex bound to p53/TFIIA/native promoter DNA. We next determined the 3D structures of TFIID/p53/TFIIA co-complexes on two distinct p53 target gene promoters via single particle cryo-electron microscopy. Strikingly, we discovered a common mode of TFIID binding to different types of promoters. Our biochemical studies showed that p53 significantly promotes TFIID's interaction with DNA. To further mechanistically dissect TFIID's enhanced promoter recognition/binding directed by p53, we examined the dynamic interaction between p53, TFIID and promoter DNA via single molecule TIRF microscopy. Taken together, our structural and functional studies elucidate how p53 facilitates TFIID-mediated transcription initiation complex assembly on different p53 target gene promoters.

2453-Pos Board B145
Single Molecule Probing of P53's Ability to Dynamically Regulate Chromatin Structure
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The tumor suppressor p53 protein is a transcriptional activator that binds to its response elements (REs) on target promoters and activates expression of a large number of genes involved in tumor suppression. Previous ChIP-seq studies indicate that p53 binds to its REs embedded in regions densely populated with nucleosomes. However, it is unknown if nucleosomes help or hinder binding of p53 to its response elements to regulate transcription.
To decipher the interaction of p53 with nucleosomes, we have utilized a combination of Next Generation Proteomics, bioinformatics, bulk biochemical and real-time single molecule FRET assays. Our proteomic assays show that p53 interacts with peptides that have strong homology to histones H2A, H2B, and H4. Biochemical assays indicate that p53 can stably interact with histone peptides in the absence of DNA. Genomic maps of p53 REs and nucleosome positions further reveal that p53 REs cluster specifically within 2 regions of the nucleosome. Furthermore these clustered p53 REs are adjacent to histone regions identified in our proteomic studies, suggesting localization of two physiologically relevant binding platforms for p53 on the nucleosome.
We have also established a single molecule FRET assay to characterize dynamic structural changes in nucleosomes with and without p53 REs. Interestingly, our studies reveal that p53 can bind to our biochemically defined nucleosomal surface and dynamically alter the structure of nucleosomal DNA independently of the presence of a p53 RE. Intriguingly, p53 has a higher affinity for its RE when incorporated into nucleosomes assembled with native DNA compared to REs on naked DNA. Thus, our studies indicate that histone/p53 contacts may enhance p53 directed transcription by creating a stable platform for p53 promoter recruitment.

2454-Pos Board B146
Substantial Nucleotide Selection Prior to Full Insertion of the Nucleotide in T7 RNA Polymerase Elongation
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Nucleotide selection is essential for fidelity control in gene replication and transcription. Previous work on T7 RNA polymerase suggests that a small post-translocation free energy stabilizes Tyr639 in the active site to facilitate the nucleotide selection. Currently, we implement atomistic molecular dynamics simulations and demonstrate that Tyr639 is indeed stabilized by ~ $2k_BT$ favorable association with the end base pair of the DNA-RNA hybrid to serve for nucleotide 'gating' from pre-insertion to insertion. Upon the nucleotide pre-insertion, a relative binding free energy above thermal fluctuation level arises against the miscoded nucleotide, primarily due to electrostatic screening from charged residues that assist the nucleotide binding. Interestingly, the pre-insertion of a right nucleotide marginally destabilizes Tyr639, while a wrong nucleotide pre-insertion substantially stabilizes Tyr639 to hinder further nucleotide insertion. The activation barrier of the miscoded nucleotide insertion under an O-helix rotation rises significantly above that of the right nucleotide. The selection against deoxyribonucleotide can be even strong and arises essentially due to steric detection from Tyr639. Our studies suggest that substantial nucleotide selection in T7 RNAP happens upon the nucleotide pre-insertion and during the insertion, prior to full insertion of the nucleotide for base pairing and chemical addition.

2455-Pos Board B147
Complete Dissection of Transcription Elongation Reveals Slow Translocation of RNA Polymerase II in a Linear Ratchet Mechanism
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During transcription elongation, RNA polymerase has been assumed to attain equilibrium between pre- and post-translocated states rapidly relative to the subsequent catalysis. Under this assumption, recent single-molecule studies proposed a branched Brownian ratchet mechanism that necessitates a putative secondary nucleotide binding site on the enzyme. By challenging individual yeast Pol II with a nucleosomal barrier, we separately measured the forward and reverse translocation rates. Surprisingly, we found that the forward translocation rate is comparable to the catalysis rate. This finding reveals a linear,

non-branched ratchet mechanism for the nucleotide addition cycle in which translocation is one of the rate-limiting steps. We further determined all the major on- and off-pathway kinetic parameters in the elongation cycle. The resulting translocation energy landscape shows that the off-pathway states are favored thermodynamically but not kinetically over the on-pathway states, conferring the enzyme its propensity to pause and furnishing the physical basis for transcriptional regulation.

2456-Pos Board B148

Localization and Tracking of Single RNA Polymerase Molecules in Live E. Coli

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Superresolution fluorescence microscopy locates and tracks the diffusive motion of single copies of RNA polymerase (RNAP) in live E. coli. On a timescale of 0.1-1.0 s, most RNAP copies separate remarkably cleanly into two states with comparable populations. The ''slow'' RNAPs move indistinguishably from DNA loci. We assign them to specifically bound copies that are initiating transcription, elongating, pausing, or awaiting termination. The ''fast'' RNAP copies act as a homogeneous population with Dfast = 0.2 μm2-s-1. These are assigned as a rapidly exchanging mixture of non-specifically bound copies and copies undergoing three-dimensional diffusion within the nucleoids. In longer trajectories of 7-s duration, we directly observe transitions between the slow and fast states, corroborating the assignments. In rapid growth conditions, for which transcription of stable RNA predominates over transcription of mRNA, the slow, transcribing RNAP copies preferentially locate at the periphery of the nucleoids, as do rrnG and RNAP ''transcription foci''. The data provide strong evidence for transcription occurring at or very near the cytoplasmic membrane, supporting the transertion hypothesis of co-transcriptional translation of membrane proteins. We compare the partitioning of RNAP states inferred from single-particle tracking with different partitioning models from the literature.

2457-Pos Board B149

Next Generation Sequencing-Based Parallel Analysis of Melting Kinetics of 4096 Variants of a Bacterial Promoter

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Promoter melting by bacterial RNA polymerase is a key step in transcription initiation. We used Next Generation Sequencing (NGS) based approach to analyze in parallel promoter melting kinetics of all 4096 sequence variants of the 6 bp −10 element of the bacterial promoter. We used NGS read count for each sequence of a promoter library containing randomized −10 sequence as an observable to determine relative enrichment of −10 element sequence variants at different time points of promoter melting reaction. The analysis reinforced the dominating role of consensus bases at positions −11 and −7 and demonstrated an enhanced preference for A at −11 among sequences exhibiting the fastest melting kinetics, consistent with the role for this base in early steps of promoter melting. However overall, somewhat unexpectedly, the T at −7 is more important then the A at −11 for efficient promoter melting. We observed a modest but significant correlation between the duplex melting energy of −10 element sequence variants and the kinetics of promoter melting. This correlation is more pronounced when the dominating base-specific interactions with RNAP are diminished. These observations indicate that promoter melting kinetics is determined by a combination of base-specific effects/interactions and sequence-dependent stability DNA duplex with the former playing a dominating role. Our data illustrate the great utility of NGS as a reliable, quantitative readout in biophysical experiments, where DNA sequence dependence of the protein activity on a large number of sequence variants could be interrogated in parallel.

2458-Pos Board B150

Direct Assessment of Transcription Fidelity by RNA Sequencing

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Cancerous and aging cells have long been thought to be impacted by transcription errors. Until now, a lack of methodology for directly assessing such errors hindered evaluation of their impact to the cells. We report a high-resolution Illumina RNA-seq method that can assess noncoded base substitutions in mRNA at 10^-4-10^-5 per base frequencies in vitro and in vivo. Statistically reliable detection of changes in transcription fidelity through ~10^3 nt DNA sites assures that the RNA-seq can analyze the fidelity in a large number of the sites where errors occur. A combination of the RNA-seq and biochemical analyses of the positions for the errors revealed two sequence-specific mechanisms that increase transcription fidelity by Escherichia coli RNA polymerase. A genome-wide assessment of transcription fidelity based on RNA-seq is also discussed.

2459-Pos Board B151

TFIIF and TFIIS Enhance the Mechanical Persistence of Transcript Elongation by RNA Polymerase II

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Mounting evidence suggests that transcript elongation in eukaryotic organisms is regulated via a variety of mechanical cues affecting entry into and exit from transient transcriptional pauses or permanent arrest. A number of accessory factors, including TFIIF and TFIIS, are implicated in the control of these processes. Here, we investigate the interactions of RNA polymerase II (RNAPII) with these factors in a single-molecule optical trapping assay. When monitoring the response of elongation complexes - containing RNAPII and combinations of TFIIF and TFIIS - to controlled mechanical loads, we find that at low force both factors are independently capable of returning arrested RNAPII to productive elongation. At high forces, TFIIF synergistically enhances TFIIS-induced transcriptional re-start but is itself incapable of re-starting transcription. Results obtained with a cleavage-defective TFIIS mutant suggest that TFIIS, in addition to its well-established role in catalyzing transcript cleavage, may relieve arrest via a second, cleavage-independent mechanism. Importantly, the factors' activities do not result in a significantly enhanced stall force, but rather promote the persistence of RNAPII in transcriptionally active states when faced with mechanical obstacles.

Our studies also uncover unexpected insights into RNAPII-intrinsic mechanisms underlying transient transcriptional pauses. Both the force-dependence of pause entry and a direct visualization of the process at near-basepair resolution reveal two distinct mechanisms: under forces opposing transcription, pauses typically originate from a backtracking event, whereas under assisting loads a backtrack-independent pause mechanism dominates. Pause duration distributions provide additional mechanistic insights, suggesting that backtracking is not a purely diffusive process, but that instead the extent of backtracking may be restricted by mechanisms intrinsic to RNAPII.

Taken together, these results provide a glimpse at how nature resolves obstacles to transcription through the concerted interactions of RNAPII with multiple accessory factors.

2460-Pos Board B152

Card Regulation of Mycobacterial Transcription Initiation

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A staggering 30% of the world's population is infected with latent tuberculosis (WHO 2010). As such, *Mycobacterium tuberculosis* (Mtb) represents a major global burden to public health, and understanding its pathology is crucial to improving therapies. CarD is a transcription factor essential for Mtb survival and pathogenesis. CarD is known to bind both RNA polymerase (RNAP) as well as DNA. Weakening CarD's interactions with RNAP improves efficacy of Rifampicin, which demonstrates the immediate clinical potential of CarD as a therapeutic target. The mechanism of CarD's interaction with mycobacterial RNAP and DNA during transcription is unknown, although ChIP-seq data shows CarD is most often located on promoter DNA throughout the genome suggesting that CarD is involved in initiation of transcription. CarD is also found at all rRNA promoters, so it is possible that CarD's essentiality arises from its role in regulation of rRNA levels during growth and latency. Therefore, to understand this essential component of Mtb pathogenesis, we aim to elucidate the mechanism of CarD-mediated regulation of transcription initiation at mycobacterial rRNA promoters.

2461-Pos Board B153

Photophysical Properties of the Spinach-DFHBI RNA Aptamer-Fluorogen Complex and its Applications to Live Cell Imaging with Improved Fluorescence Signal

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The use of aptamer-fluorogen complexes is an emerging strategy for RNA imaging. Despite promise for cellular imaging and sensing, the low fluorescence